The role of Ischemia reperfusion damage on renal transplant, what are the new treatments?

Ischemia reperfusion damage usually occurs after renal transplantation. These injuries can stimulate the innate immune system, trigger an inflammatory response and ultimately activate the adaptive immune system. These events may result in rejection, graft fibrosis and chronic allograft nephropathy.

Different mechanisms contribute to innate immune system activation following ischemia reperfusion injury in renal transplants. Some of these mechanisms are known and described by investigators while the remaining are still unknown.

To clarify the precise mechanisms underlying the innate immune system activation and rejection progression helps us to plan therapeutic protocols to reduce immunologic responses to ischemic events and to improve the graft function and outcome. In this review, we will discuss how innate and adaptive immune systems are activated during an ischemic insult and thereafter discuss related therapeutic interventions to block the activating pathways.

Keywords: Ischemia; Renal transplantation; Reperfusion.

Introduction

Varied degrees of ischemia reperfusion damage usually occur after renal transplantation. Graft ischemic damage in renal transplantation can occur during surgery and/or can be due to suboptimal graft perfusion during the intra or post-operative period. Ischemia reperfusion injury is the most common cause of delayed graft function (DGF) and increases the immunogenicity of graft and thereby increases the rate of acute rejection and chronic allograft nephropathy in recipients. The results have revealed that unrelated living donor grafts (even with a significant major histocompatibility complex mismatch) have a better survival than HLA matched deceased donors (DD). Additionally,
chronic graft nephropathy has been demonstrated in transplants between identical twins. These observations suggest the contribution and importance of antigen independent risk factors such as ischemia on the graft survival [1-6]. Ischemia reperfusion injury is a biphasic phenomenon that can damage the graft in both processes. Ischemia initiates the injury and reperfusion exacerbates the ischemic injury by inflammatory responses induced by endothelial factors oxygen free radicals and leukocytes. Although reperfusion is essential to the survival of the ischemic graft, it triggers events that lead to renal cell apoptosis and necrosis. The mechanisms of the ischemic damage to renal transplant are similar to ischemic nephropathy in patients with acute tubular necrosis. It is important to know that the ischemic damage can predispose the graft to acute rejection episodes, and subsequent chronic allograft nephropathy and graft loss, if it occurs early after renal transplantation. In this review, we will discuss the association between ischemia reperfusion damage to the graft and the occurrence of acute rejection and chronic allograft nephropathy. We thereafter discuss the therapeutic interventions that reduce the ischemic damage to achieve a better graft survival.

**Ischemia and acute rejection association**

Ischemia reperfusion injury can stimulate the innate immune system and consequently trigger an inflammatory response in the body after transplantation. Additionally, increased expression of class I and II MHC is reported in the ischemic graft. The innate immune system activation can also initiate the adaptive immune system response. This interaction between innate and adaptive immune systems might be an implication of the association of acute and chronic rejection with ischemia reperfusion injury [7,8]. It is believed that inflammation due to ischemic injury is a critical factor to initiate rejection [9-13]. The cellular events that occur in ischemic reperfusion injury after renal transplantation are described below.

1- The activation of the innate immune system in ischemic reperfusion injury

The innate immune system including monocytes, eosinophils, macrophages, neutrophils and natural killer cells is an important protective system against infections. Major components of the innate immune system participate in the pathogenesis of ischemic reperfusion injury in the kidneys[14]. At first, ischemia activates the innate immune system. Signals that activate the innate immune system consist of factors released, synthesized and secreted from the injured endothelial and epithelial cells of renal tubules. In addition, injured cells activate the innate immune system and decrease the anti-inflammatory factor expression [14]. One of these signals is the TLR/IL-1R family. These signals activate the innate immune system through toll like receptors (TLRs) which foster the recruitment of inflammatory cells [15]. Experimental studies have shown that the expression of both TLR-2 and TLR-4 are increased during ischemia reperfusion in tubular epithelial cells in the kidney [16,17]. TLR2 is an important initiator of inflammatory processes and its inhibition leads to treatment or prevention of the ischemia reperfusion damage [18]. TLR4 induces the expression of endothelial chemokines. MyD88 is a proximal tubule...
protein that translates TLR and IL-1R signals and promotes nuclear localization of NF-κB. In MyD88 deficient mice, the maturation and migration of dendritic cells is failed and consequently T helper cell dependent alloimmune response are reduced [19-24].

Activation of toll like receptors leads to the expression of endothelial adhesion molecules and production of cytokines and chemokines. Reactive oxygen species (ROS) and complement activation also increase the synthesis and release of cytokines and chemokines. These proinflammatory cytokines include interferon, monocyte chemotactic protein 1 (MCP-1), IL1, IL6, TNF-a, IL-8 and macrophage inflammatory protein (MIP)-2 [25]. Endothelial adhesion molecules also consist of selectins, intracellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM), and PAF (platelets activating factor). Additionally, renal ischemia induces the activation of transcription factors like NF-κB, heat shock factor-1, and hypoxia-inducible factor-1 (HIF-1) [26,27]. These factors induce inflammation by production of IL-1a, IL-6, IL-8, cyclooxygenase (COX2), tumor necrosis factor (TNF)-α, or interferon (IFN)-γ, MCP-1, RANTES, and endothelial adhesion molecules [28-31].

Activation of the alternative complement pathway is also associated with ischemia reperfusion injury. The production of C3 is increased by tubular epithelial cells injured by ischemia. Intracellular calcium is increased during the reperfusion period, and then leads to cleavage of the cell surface complement inhibitory proteins. This event causes uninhibited activation of the alternative complement pathway [9,32]. C5b- 9 complex (membrane attack complex, MAC) and C5a are also associated with acute ischemic damage to the kidney [33,34]. Decay-accelerating factor (DAF, CD55), which blocks the formation of the membrane attack complex, is demonstrated to attenuate injury in an acute kidney ischemic injury model [35].

Koo et al performed a study in which biopsy was obtained from transplants of DD (n = 55) and living/related (n = 11) kidney allografts at two time points, preperfusion: after donor nephrectomy and post perfusion which is approximately 20 to 40 minutes after revascularization of the graft. They observed an increased expression of adhesion molecules (P-selectin, E-selectin, ICAM1, and VCAM1), neutrophil infiltration and platelets deposition in the graft after revascularization which were more prominent in DD kidney grafts. Subsequently, the migration of neutrophils causes local tissue destruction by releasing proteases and oxygen-free radicals. They demonstrated a significant association between immunologic changes including the presence of neutrophils and platelets in glomeruli and subsequent graft function like the serum creatinine level at 3 and 6 months after transplantation [36]. It appears that macrophages, neutrophils, natural killer (NK) cells, and dendritic cells are important cells activated in innate immune responses. The role of macrophages is not completely defined. These cells may play a protective role in ischemia reperfusion but several recently performed studies have exhibited that macrophages contribute to the development of fibrosis in the recovery phase of acute ischemic kidney [27,37]. Neutrophils play important roles in the innate immune system. These cells
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phagozyte, plug the renal microvasculature and release the proteases and free oxygen radicals. The role of natural killer cells is not known completely, but it is suspected that they play a role in inflammation by secreting cytokines that activate neutrophils and macrophages [14]. They also secret INF-γ which is an important factor in the activation of dendritic cells.

The ischemia reperfusion damage to the graft causes the residents dendritic cells to release TNF alpha and the migration of recipient dendritic cells to the graft, and consequently predisposes the graft to acute rejection [6,38]. It is reported that the predominant TNF secreting cells in the early period of ischemia reperfusion are the resident dendritic cell [38]. Schlichting et al hypothesized that dendritic-endothelial cells interaction is the first step in the renal ischemia injury that mediates graft rejection [39].

2-Innate like immune response to ischemia reperfusion damage

Innate like lymphocytes are a subset of lymphocytes with limited expression of receptors and without clonal expansion. These lymphocytes are composed of natural killer T cells, intraepithelial γδ T cells, and B-1 subset of B cells (B-1 cells). Natural killer T cells present CD40 on their surface. The traffic of these cells after kidney ischemia begins 3 hours after injury and disappears 24 hours after injury. NK T cells secret cytokines such as IL-4, IL-10, and IFN-γ and then exacerbate inflammation. Other unconventional T cells (intraepithelial γδ T cells) are a subset of T cells with receptors consisting gamma delta chains instead of alpha beta chains. Release and production of diphosphoglyceric acid, isopentenylpyrophosphate and heat shock proteins by ischemic injured renal epithelial cells result in the activation of intraepithelial γδ T cells or gamma-delta T cells. Experimental studies have demonstrated that the degree of renal tubular injury after ischemia reperfusion is reduced in γδ T cell-deficient mice as well as αβ T cell-deficient mice [40,41]. The role of B1 cells in ischemia reperfusion induced acute kidney injury is unknown, but some reports have shown the role of these cells and the secreted IgM in ischemia injuries [42].

3- Activation of adaptive immune system in ischemia reperfusion damage

Activation of complement, cytokines and chemokines, and toll like receptors is predicted to link innate and adaptive immune systems. The co-stimulation pathway and CD4 T cells and adaptive immune response components have been identified as the mediators of kidney injury in ischemia reperfusion injury [14, 17, 38, 43-46]. Activation of TLR/IL-1R on resident dendritic cells due to ischemia reperfusion injury promotes their maturation resulting in up regulation of MHC class II, co-stimulatory molecules and lymphoid receptors [15,17,38]

Loverre et al evaluated T helper cell phenotype in patients with DGF. They found that Th1 phenotype cells were increased significantly within the graft in patients with DGF and suggested that this event could reveal a link between ischemia (DGF) and acute rejection [29]. An experimental murine model demonstrated that Th1 cells were pathogenic in contrast to Th2 cells that were protective in ischemia reperfusion injury [47]. Thus, blockade of Th1 cells and activation of Th2 cells may be protective and lead to tolerance.
However, ischemia activates Th1 cells and subsequently the rejection pathway. On the other hand, it has been shown that ischemic injury increases the major-histocompatibility-complex (MHC) class I peptide–related sequence A (MICA) antigens on endothelial surfaces. These events result in the activation of natural killer cells and CD8 T cells [48]. Ischemia also induces the co-stimulatory pathway: the release of cytokines like TNFa during ischemia induces the expression of B7 in the ischemic graft. This event leads to rejection rather than tolerance [49,50]. Other signals representing a barrier tolerance are TLR. Some experimental studies have demonstrated that TLR agonists reduce the cardiac allograft acceptance by preventing the tolerance of the graft [22, 48]. Some negative regulators of TLR/IL-1R have been identified. One of them, toll-IL-1R8 (TIR8), is a key regulator of allogenic immune response and a powerful tolerogenic factor. In an experimental study, TIR8-deficient (Tir8−/−) mice were associated with acute rejection [15].

**Ischemia and chronic allograft nephropathy association**

Ischemic injury and its consequent renal mass reduction lead to chronic graft dysfunction which may be due to hyperfiltration and infiltration of T cells and macrophages, the release of biological mediators from them, and ultimately fibrosis. These mediators such as IL-1, TNF- TGF-β1, endothelin-1, and nitric oxide stimulate proliferation of arterial smooth muscle cells, glomerular mesangial cells, and fibroblasts. Among these mediators, TGF-β1 is a key fibrogenic cytokine. Other factors are also involved in the development of chronic graft dysfunction including PAF and angiotensin. Some studies have shown the effect of chemokines in angiogenesis, graft arteriosclerosis, and fibrosis [51,52].

**In summary**, ischemic injury recognition by the immune system triggers rejection thorough the following ways:

1. Ischemia induces activation of endothelium to express adhesion molecules and produce cytokines and chemokines
2. Ischemia induces translocation of leukocytes into the interstitium
3. Ischemia induces activation of transcription factors such as nuclear factor- B and c-jun via stress-activated protein kinases [53,54,55].
4. Activation of the alternative pathway of the complement system
5. Activation of the coagulation system
6. Ischemia induces the co-stimulatory pathway.
7. Ischemia induces the expression of CD40 on APC; the interaction between CD40 and CD154 on the T cells increases the expression of adhesion molecules and release of chemokines in the endothelium [56].
8. Renal tubular cell injury due to ischemia activates unconventional T cells. Unconventional T cells include natural killer cells with CD4 on their surface and T cells with receptors consisting of the gamma delta chains instead of alpha beta chains.
9. Ischemia induces up-regulation of MHC class I and II antigens in the graft.
The management of patients with ischemic graft is supportive. We need therapeutic strategies to focus on the cellular level in order to attenuate the cellular and molecular events participating in the ischemia reperfusion damage. Administration of these medications to recipients before and shortly after transplantation may diminish cellular damage that occurs during ischemia reperfusion and ultimately may improve the graft outcome. We also need long-term therapeutic interventions in patients with a history of delayed graft function to prevent chronic allograft nephropathy due to the ischemic immunologic damage to the graft and kidney mass reduction.

1. Using correct surgical techniques
2. Minimizing cold and warm ischemia times
3. Using cold preservation solutions with antioxidant compounds in them [57-59].
4. Optimizing graft perfusion during the intra and post-operative period.
5. Using vasodilators which promote the synthesis of nitric oxide and reduce vasoconstriction of calcineurin inhibitors [60,61] such as PGE and dopamine.

However, in a trial study, PGE1, dopamine and high dose furosemide were administrated to 100 patients and the results of primary graft function were compared with the control group. There were no differences between these groups in the incidence of the delayed graft function and therefore, this study did not show the effectiveness of this regimen in reducing DGF [62].

6. Antioxidants: antioxidants may remarkably influence the degree of ischemia reperfusion damage by reducing the inflammatory response in the allograft. Treska et al showed that the use of intravenous antioxidants like intravenous multivitamin and immunosuppressive medications in recipients one hour before transplantation may have a considerable impact on the immediate graft function after kidney transplantation from non-heart beating donors [63]. Danilovic A et al assessed the effects of N-acetylcysteine (an antioxidant substance) on the early graft function of deceased renal transplantation. N-acetylcysteine administration was shown to improve the early graft function (3 months and one year after transplantation) of deceased donor renal transplantation by reducing the oxidative stress [64]. The application of oxygen radical scavengers, such as superoxide dismutase, has been shown to be beneficial in reducing ischemic damage [58].

7. Immunosuppressive medications: In patients with DGF, basiliximab [65] or antilymphocyte antibodies can be started post-operatively to delay the introduction of calcineurin inhibitors. The protective effect of rabbit anti-rat thymocyte immunoglobulin (rATG) was recently evaluated in a rat model of ischemic injury after kidney transplantation. It was suggested that the administration of rATG, two hours before transplantation to recipient animals reduced ischemic injury to the graft such as reduction in tubular apoptosis and the amount of infiltrated macrophages, CD4(+) , CD8(+) T cells and LFA-1(+) cells in histopathology. They found that the administration of rATG at the time of reperfusion did not have these effects[66]. In a human study, single-dose thymoglobulin (TG) induction (1.5 mg/kg) in living donor renal transplantation resulted in higher patient and graft survival without increasing the risk of infection or malignancy [67]. It has been believed that thymoglobulin reduces leukocyte rolling as well as leukocyte-
chemotaxis or chemokine receptor expression. Thus, one of the major effects of thymoglobulin is the prevention of leukocyte traffic. TG also depletes T cells, impairs the function of the non depleting T cells and prevents the memory T cell migration [68].

Another immunosuppressive agent that can prevent rejection during recovery from ischemia in renal transplants through alteration of T regulatory cells trafficking. MMF can modify epithelial proliferation during the healing phase of an ischemic insult in renal transplants [69].

8. Treatments targeting the innate immune system:

• Inactivation of TLR2 [70,71] and TLR4 [72]: Recent studies have shown that defective TLR-4 signaling leads to better graft function and lower expression of proinflammatory cytokines [73]. However, the deletion of TLR2 and TLR4 together does not result in additional benefits in comparison with single deletion in one of them [74]. OPN-305 is a humanised IgG4 monoclonal antibody against toll-like receptor 2 which is under investigation as a treatment for the prevention of delayed graft function following renal transplantation. In a recent experimental study, it was demonstrated that the inhibition of toll like receptor 4 with eritoran (a synthetic toll like receptor 4 antagonist) reduced the ischemic injury to the graft. In this study, the tubular loss and monocyte infiltration were significantly lower in rats under treatment with eritoran [75].

• Inactivation of adhesion molecules [76,77], Therapies that decrease the chemokine production [78] and cytokine inhibitors: Treatments inhibiting the adhesion molecules involved in neutrophil translocation like selectins, ICAM 1, and CD11/CD18 play a protective role in experimental studies [79,80,81]. Two human trials about the application of ICAM 1 blocking agents in prevention of DGF in renal transplantation have showed contradictory results. One of them exhibited the beneficial effect of this treatment on the rate of DGF and acute rejection [82], but the other failed to demonstrate the beneficial role of anti ICAM 1 in the recipients of DD renal allograft. Vincenti F et al assessed the use of efalizumab, a humanized IgG1 anti-LFA-1 antibody, in a calcineurin inhibitor free protocol. They found The good tolerability of this medication, but they did not assess its influence on graft ischemia and long term function [83]. Experimental studies have reported the beneficial effects of PAF blockade and alpha- melanocyte-stimulating hormones, as the inhibitors of IL-8 and ICAM-1, in the reduction of ischemic injury [78,84]. A recent study in a large animal model revealed that carbon monoxide administration intraoperatively for 1 hour reduced acute tubular necrosis, apoptosis, P-selectin, MCP-1 and heat shock proteins expression and enhanced proliferative repair as measured by phosphorylation of retinol binding protein and histone H3 [85]. The use of anti inflammatory cytokines such as IL13 [86] has also been suggested to reverse the inflammatory process but more experience is required with these therapies [25]. Some studies have demonstrated the important role of TNF alpha inhibitors to improve the ischemic damage to the graft as well as to prevent and treat the acute rejection episode [87].

• Inhibitors of the alternative complement
pathway: In practice, the use of the complement inhibitor sCR1 reduces the inflammation and infarction size in patients with ischemic heart disease [88]. An experimental study revealed the protective effects of monoclonal antibodies to factor B in the ischemia reperfusion damage [89]. C5a receptor antagonist also represents renal protective effects by the reduction of chemikines and consequently neutrophil infiltration [90,91]. A clinical trial is currently being performed about the use of eculizumab for the prevention of delayed graft function in deceased donor kidney transplants in adults but their results are not yet available.

9. Apoptosis inhibition in the damaged tissue and avoidance of medications which promote apoptosis like mTOR inhibitors: mTOR inhibitors are not suggested for use in the early phase of transplantation, especially in the presence of ischemia reperfusion injury, because they promote apoptosis [92,93]. In contrast to apoptotic properties of rapamycin, this medicine has some inhibitory effects on the dendritic cells. Thus, rapamycin can induce some tolerogenic effects and prevent the ischemic damage to the graft by dendritic cells suppression [94].

Zinc finger protein A20 is an anti-apoptotic and anti-inflammatory molecule that mediates the regulation of apoptosis receptors and inhibition of NF-κB and TLR-4 signaling [95]. Overexpression of protein A20 has been shown to decrease ischemia reperfusion injury in experimental studies, but no clinical study has been conducted about protein A20 overexpression in transplantation. QPI-1002 (I5NP) “a synthetic siRNA” inhibits the expression of the pro-apoptotic protein p53. It has been used as prophylaxis of DGF after deceased donor renal transplantation. Phase 1 study in deceased renal transplantation has been completed and the next phase is being conducted.

10. Treatment inhibiting the adaptive immune response: The blockade of IL16, “a strong chemotactic cytokine for CD4 T cells” results in less CD4 T cells infiltration and consequently prevents the ischemic injury. Blockade of the B7-CD28 pathway by CTLA4Ig reduces the acute and chronic allograft nephropathy following graft ischemia [96]. In a rat model, the administration of CTLA4Ig during the first week after ischemia reperfusion damage reduced proteinuria in the long term [97]. It is predicted that the blockade of the costimulatory pathway may lead to tolerance.

11. TGF-β1 bio-activity blocking agents

12. Growth factors [98] such as hepatocyte growth factor: The protective effects of hepatocyte growth factor to reduce renal dysfunction and improve renal proximal tubules have been shown by the Miller’s experimental study in a rat model of ischemic renal injury [99]. Nakatani et al also performed an experimental study in which hepatocyte growth factor was added to the preservation solution of renal transplant. They showed that hepatocyte growth factor improved the graft hemodynamic state [100].

13. Atrial natriuretic peptide [101]: Atrial natriuretic peptide has been shown to inhibit the production of inflammatory mediators. It seems that human ANP is effective in reducing renal ischemia by reducing neutrophil activation. This effect of ANP is demonstrated by some experimental studies [102,103].

14. High dose erythropoitin: An experimental study demonstrated that up
regulation of erythropoietin decreased the severity of injury due to ischemia reperfusion. Another experimental study in a rat model suggested that the administration of erythropoietin inhibited apoptosis and helped to preserve the graft function in marginal donors [104]. Danilo Fliser from Hannover Medical School performed a clinical trial to assess the effect of high dose erythropoietin B on the graft function one month after kidney transplantation. No difference in the GFR at 1 month post transplantation and adverse events was observed between patients who received erythropoietin and those who did not [105].

15. BAY 12-9566 is a matrix metalloproteinase inhibitor. Its administration early after transplantation (day 0-10) has been shown to reduce tissue damage, resulting in a better graft outcome ultimately [106].

Conclusions
Ischemia reperfusion injury activates innate immunity. Although T cells have critical roles in acute rejection episodes, the innate immune system activation and up regulation of pro-inflammatory mediators which is induced by ischemia reperfusion injury occur before the T cell response and then play some roles in the activation of adaptive immunity and acute rejection. The innate immunity activation is important for optimal adaptive immune response and resistance to tolerance induction. However, the precise mechanism underlying the role of the adaptive immune system in ischemia reperfusion damage to the graft is not well known and more investigations are required in this field. Also, the investigations on agents blocking the innate immune response are probably important in planning more advanced treatments.

Conflict of Interest
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