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Lymphocytes and ischemia-reperfusion injury

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Abstract

Ischemia reperfusion injury (IRI) is a common and important clinical problem in many different organ systems, including kidney, brain, heart, liver, lung, and intestine. IRI occurs during all deceased donor organ transplants. IRI is a highly complex cascade of events that includes interactions between vascular endothelium, interstitial compartments, circulating cells, and numerous biochemical entities. It is well established that the innate immune system, such as complement, neutrophils, cytokines, chemokines, and macrophages participate in IRI. Recent data demonstrates an important role for lymphocytes, particularly T cells but also B cells in IRI. Lymphocytes not only participate in augmenting injury responses after IRI, but could also be playing a protective role depending on the cell type and stage of injury. Furthermore, lymphocytes appear to be participating in the healing response from IRI. These new data open the possibility for lymphocyte targeted therapeutics to improve the short and long term outcomes from IRI.

1. Introduction

Ischemia-reperfusion injury (IRI) is a common and important clinical problem in many different organ systems. Ischemia-reperfusion injury is seen in myocardial infarctions, strokes, acute kidney injury, shock liver, mesenteric ischemia, and systemic shock. Ischemia-reperfusion injury is common in all deceased donor solid organ transplants. Both clinical and experimental data demonstrate that transplant IRI has deleterious short- and long-term effects, manifesting as increased episodes of acute rejection and chronic allograft dysfunction [1]. During solid organ transplantation, both warm and cold ischemia are often unavoidable but can be minimized. To date, no specific therapy exists for the prevention or treatment of IRI. Recently, experimental models examining the pathogenesis of IRI have revealed new mediators, including lymphocytes. This brief review will focus on the new data implicating lymphocytes in IRI.

2. Pathogenesis of ischemia-reperfusion injury

Cerra et al [2] were among the first to describe reperfusion injury in 1975 in a canine model. They examined myocardial pedicles to detect the extent of reperfusion injury and found that increased ischemic times were associated with increased subendothelial hemorrhagic necrosis. The same group then looked retrospectively at 125 patients who had undergone aortic valve replacement and, specifically, 25 patients who died after the procedure. They found 5 of the 25 patients had subendothelial hemorrhagic necrosis of the myocardium on autopsy. All five of the patients had been subjected to greater than 70 minutes of cardiopulmonary bypass time, i.e., increased ischemic time. They concluded that IRI was the most common cause of myocardial infarction and death following aortic valve replacement.

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Ischemia-reperfusion injury is now recognized as a highly complex cascade of events that includes interactions between vascular endothelium, interstitial compartments, circulating cells, and numerous biochemical entities. Inflammation is known to be a key mediator of IRI and considerable data exist demonstrating the significance of the innate immunity [3–5]. Parenchymal damage occurs from both direct microvascular dysfunction from hypoxia and the subsequent inflammatory response. Acute ischemia leads to oxygen deprivation and adenosine triphosphate depletion resulting in direct parenchymal damage through tissue necrosis. Apoptotic damage can also occur in a low temperature, low adenosine triphosphate milieu. Upon restoration of blood flow to the ischemic tissue, a “no-reflow” phenomenon occurs [6–8]. Capillaries and microcapillaries are not perfused, potentiating further tissue damage. The mechanisms of failed capillary perfusion include endothelial cell swelling, capillary narrowing due to interstitial edema, and intravascular sludging from hemoconcentration and capillary vasoconstriction that is mediated by many chemokines and cytokines. The sequelae of events described above leads to direct microvascular dysfunction and parenchymal damage.

Acute ischemia also triggers a vigorous immune response. Acute ischemia causes activation of endothelium resulting in increased permeability and increased expression of adhesion molecules. Ischemic endothelial cells acquire an adhesive, thrombogenic surface. These primed endothelial cells are more adhesive, and upon reperfusion, inflammatory cells attach to the endothelium [9]. Reactive oxygen species, cytokine, chemokines and adhesion molecules are generated, secreted, and released, augmenting the inflammatory reaction [3–6,10–14]. The combination of vascular permeability and increased cellular signaling augment the recruitment and infiltration of circulating leukocytes into the postischemic tissue. This inflammatory response has been shown in both experimental models and human data to result in tissue destruction and organ dysfunction.

The inflammatory response to acute ischemia in the classic model is predominantly an innate immune response. Polymorphonuclear cells have been shown to be the major leukocytes found in necrotic tissue following ischemic injury. Neutrophils are thought to be the early cellular mediator of local microvascular changes and parenchymal damage. Monocytes and macrophages infiltrate later in IRI and likely extend the early injury phase [15]. The complement system is also activated during IRI and contributes to tissue destruction. B and T cells constitute the major mediators of the adaptive immunity and were not thought to play a role in the pathogenesis of IRI. Recent data suggests that this is not the case and clearly lymphocytes have a significant role in the response to IRI in a variety of organ systems.

3. T cells as mediators of IRI

Numerous studies involving a variety of organ systems have identified T-cell trafficking into organs following IRI. Previously thought to be “passive observers” in the inflammatory response, there is now an overwhelming body of literature in different organ systems demonstrating the role of T cells as direct mediators of IRI. T cells have been identified by immunohistochemistry in the postischemic brain within 24 hours of reperfusion and localize to the stroke boundary zone near blood vessels [16,17]. Yilmaz et al [18] demonstrated that Rag1^{-/-} mice, deficient in both T and B cells, subjected to middle cerebral artery occlusion had significantly reduced cerebral infarct size and neurologic damage compared to WT control mice. Rag1^{-/-} mice reconstituted with splenocytes from wild-type (WT) mice were no longer protected from injury. In the same study, the authors showed that mice deficient of CD8⁺ T cells, CD4⁺ T cells, and interferon (IFN)- γ had reduced volume of cerebral infarct compared to WT mice as well. Hurn et al [19] examined mice with severe combined immunodeficiency (SCID), lacking both B and T cells, 22 hours after middle cerebral artery occlusion. They found that compared to WT mice, there was a greatly reduced infarction size in the SCID mice. This also demonstrated that T cells and possibly B cells were mediators of brain IRI.

T cells have been demonstrated to mediate IRI in the lung. Using a syngeneic lung transplant model in Lewis rats, de Perrot et al [20] demonstrated that recipient CD4⁺ T cells infiltrated lung grafts within 1 hour of reperfusion and up-regulated the expression of CD25 over the ensuing 12 hours. When compared to T cell-deficient nude rats (rnu/rnu), WT Lewis rats had decreased oxygenation and increased peak airway pressures indicating more severe injury in the mice with functional T cells. There were significantly higher levels of IFN- γ in transplanted lung tissue of recipient WT rats compared to nude rats. To confirm that T cells were key mediators of injury, nude rats that were reconstituted with T cells from heterozygous rats. These reconstituted nude rats developed the injury pattern seen in WT rats after 12 h of reperfusion. Geudens et al [21] also demonstrated a pathogenic role of T cells in a lung IRI model in SCID mice. SCID and control mice underwent 90 minutes of ischemia. After 4 hours of reperfusion, there was a significant decrease in neutrophils and interleukin (IL)-1 β in SCID mice compared to controls.

T lymphocytes have also been demonstrated to be pathogenic during myocardial IRI. Yang et al [22] evaluated myocardial infarct size in RAG1^{-/-} mice and controls following 45 minutes of left anterior descending coronary artery occlusion. RAG1^{-/-} mice had significantly smaller infarct size compared to that of control mice. After reconstitution of RAG1^{-/-} mice by adoptive transfer with CD4⁺ T cells, the infarct size of the reconstituted RAG1^{-/-} mice was significantly greater than that of the RAG1^{-/-}. RAG1^{-/-} mice reconstituted with CD4⁺ T cells from IFN- γ ^{-/-} showed no increased myocardial infarct size indicating that the cytokine IFN- γ may be an important mediator of IRI. They also examined T-cell depletion and its effect on myocardial infarct size. CD4⁺ depleted mice, but not CD8⁺ depleted mice had a significantly decreased infarct size compared to control mice again implicating CD4⁺ T cells' deleterious role in IRI.

Intestinal IRI has also been shown to be, in part, mediated by T lymphocytes. Shigematsu et al [23] occluded the superior mesenteric artery of both WT and SCID mice for 45 minutes, followed by 30 or 360 minutes of reperfusion. They examined both extravasation of albumin and T-cell adhesion following reperfusion. In the SCID mice, there was a significant decrease in intestinal leakage of albumin compared to the WT at 30 minutes. Restoration of the WT levels of albumin extravasation was seen in SCID mice reconstituted with T cells from WT mice. T-cell adhesion was significantly increased after 6 hours of reperfusion but was not significantly increased 1 hour after reperfusion. This suggests that T cells can have a pathogenic effect during IRI even in the absence of adhering to vascular endothelium. Tissue myeloperoxidase activity was also significantly decreased in the SCID mice when compared to WT, suggesting a role for T cell recruitment of neutrophils. Recruitment of neutrophils early in the reperfusion injury could account for endothelium damage rather than direct cytotoxic effects of the T cells themselves.

Experimental data also have implicated T cells as mediators of hepatic IRI. Zwacka et al [24] demonstrated the pathogenesis of CD4 cells in liver IRI. BALB/c (WT) and athymic (nu/nu) mice were subject to lobar hepatic IRI. Although there was no significant difference between the 2 groups the acute injury phase (3–6 hours), the nu/nu mice had significantly reduced injury 16–20 hours post ischemia, both serologically and histologically, when compared to the WT mice. In vivo depletion of CD4⁺ T cells in WT mice also resulted in decreased serologic and histopathologic injury, whereas adoptive transfer of CD4⁺ T cells into the nu/nu mice restored the injury. In vivo depletion of CD8⁺ T cells had no effect on the injury pattern seen. Khandoga et al [25] used a warm ischemia model of hepatic IRI. They showed that compared to WT mice, CD4^{-/-} mice had significantly decreased hepatic injury and improved postischemic perfusion of the hepatic sinusoids. The putative mechanism of CD4-mediated pathogenesis was thought to be interaction with platelets. By infusing fluorescent-labeled platelets after IRI, the authors tracked platelet endothelial cell interactions. CD4^{-/-} mice had significantly less platelet

endothelial cell interaction in the hepatic sinusoids compared to WT mice. Blocking the platelet receptor, GpIIb/IIIa, also significantly reduced platelet accumulation in the sinusoids.

Many studies have been performed demonstrating the pathogenesis of T cells in renal IRI. Rabb et al [26] studied renal IRI in mice deficient of CD4 and CD8 T cells (CD4/CD8^{-/-}). At 2 days post IRI, CD4/CD8^{-/-} mice had significantly improved renal function, significantly less neutrophil infiltration and markedly decreased tubular atrophy scores compared to WT controls. This suggests a protective effect of T cell depletion on renal IRI. Burne et al [27] examined the effects of renal IRI on T cell-deficient mice (nu/nu), CD4-deficient mice (CD4^{-/-}), and CD8-deficient mice (CD8^{-/-}). These mice were subject to 30 minutes of warm ischemia. Both nu/nu mice and CD4^{-/-} mice had significantly less histopathologic and serologic injury compared to WT mice. CD8^{-/-} mice had similar functional and structural injury patterns as seen in WT mice. Reconstitution of nu/nu mice by adoptive transfer with T cells from WT mice restored the injury phenotype as did adoptive transfer of isolated CD4⁺ T cells into the CD4^{-/-} mice. Transferring CD4⁺ T cells from CD28-deficient mice was not sufficient to restore the injury phenotype in nu/nu mice, nor was transferring CD4⁺ T cells from IFN- γ -deficient mice into nu/nu mice. This suggests that both costimulation with CD28 and a T_H1 milieu of CD4⁺ T cells are important contributors to renal IRI.

Savransky et al [28] examined the effect of T cell receptor (TCR) depletion on renal IRI. Knockout $\alpha\beta$ -TCR and $\gamma\delta$ -TCR mice were subjected to warm IRI. The $\alpha\beta$ -TCR-deficient mice were protected from serologic kidney injury 24 hours after ischemia-reperfusion, as measured by serum creatinine, compared to WT mice. Both $\alpha\beta$ -TCR and $\gamma\delta$ -TCR-deficient mice had significantly decreased histopathologic renal injury compared to WT mice. Hohegger et al [29] found similar protective effects of TCR deficiency. They found both $\alpha\beta$ -TCR and $\gamma\delta$ -TCR-deficient mice had significantly decreased serologic and histopathologic renal injury compared to WT mice 72 hours following ischemic reperfusion. There was also a significant decrease in infiltrating CD4⁺ T cells in the TCR-deficient mice compared to WT mice.

4. Indirect evidence of T-cell involvement

There exists a large collection of indirect evidence implicating the T cell as a pathogenic mediator of ischemia-reperfusion injury (Table 1). Many therapies that modulate the immune response and, specifically, the T-cell response have been used in experimental models. Copolymer-1 (Cop-1) is a random polymer of amino acids that is a potent inducer of the T_H2 regulatory T cell by cross reacting with myelin basic protein (MBP) and producing suppressor T cells specific to MBP. Copolymer-1 has been previously shown to suppress experimental allergic encephalomyelitis by blocking the response to MBP. Ibarra et al [30] looked at the effects of Cop-1 in a rat model of stroke. Animals were subjected to middle cerebral artery occlusion for 2 hours; half of the animals were treated with Cop-1 and the others with saline. Rats treated with Cop-1 had significantly improved neurologic function 7 days after IRI as well as significant histologic preservation of neural tissue when compared to the saline group. Although the mechanism was not directly explored in this study, previous studies indicate that Cop-1 acts by inducing T_H2 and even T_H3 T cells which then create an anti-inflammatory milieu by releasing cytokines such as IL-4, IL-10, and transforming growth factor (TGF)- β [31]. Altering the T-cell response from a T_H1 to a T_H2/T_H3 predominance following middle cerebral artery occlusion likely accounts for the neuroprotective effects observed with Cop-1 treated rats.

Carbon monoxide (CO) can have protective effects in a variety of injury models. Specifically, CO has been cytoprotective in IRI transplant models in multiple organ systems including the heart, lung, kidney, and intestine [32–36]. Mechanistically it is thought that CO may have some anti-inflammatory properties accounting for its beneficial effects. Nakao et al [33] used a

cardiac transplant model to study the cytoprotective effects of CO. Allografts that were treated with low-dose CO had improved long-term survival after transplantation. These allografts showed decreased infiltrating CD3⁺ T cells. There was reduced expression of pro-inflammatory cytokines as early as 3 hours after IRI that was again seen 28 days post IRI. These data suggest CO can alter the T_H1 response to IRI and may ameliorate the injury response. Other studies have suggested that CO may suppress T-cell proliferation and IL-2 expression through the extra-cellular signal-regulated kinase–MAPK pathway or through the p21 Cip 1–dependent caspase activity [37,38].

Calcineurin inhibitors have been extensively used in models of IRI. Calcineurin activates the transcription of IL-2, which, in turn, stimulates the growth and differentiation of the T-cell response. FK506, a calcineurin inhibitor, has been shown to reduce cerebral infarct size in both rat and mouse models of brain IRI [39,40]. In a liver model of IRI, FK506 was also found to be protective by decreasing leukocyte adhesion through down regulation of intercellular adhesion molecule (ICAM-1) and P-selectin [41]. Two separate models of mesenteric IRI have shown that FK506 and/or cyclosporine have been protective of IRI; this may occur through suppression of endothelin-1 expression [42,43]. Both FK506 and cyclosporine have been shown to improve both histopathologic and neurologic outcomes 24 and 48 hours after IRI in a rabbit spinal cord model; the mechanism of this protection is thought in part due to the anti-inflammatory properties of these drugs [44]. Pretreatment with cyclosporine or FK506 also ameliorates lung IRI likely occurring through the down-regulation of proinflammatory cytokines including IL-2, tumor necrosis factor (TNF) α , and IFN- γ [45].

Antithymocyte globulins (ATGs) are used in induction immunosuppression therapy for organ transplantation and to treat acute rejection after transplantation. Antithymocyte globulins induce apoptosis and may inhibit leukocyte adhesion in peripheral T-lymphocytes. In a skeletal muscle model of IRI, pretreatment with ATG reduced circulating leukocytes, reduced infiltrating leukocytes in both muscle and vasculature, and showed decreased histologic tissue damage when compared to control animals [46]. The proposed anti-inflammatory mechanisms of ATGs include direct effects on leukocyte adhesion molecules and proinflammatory molecules and/or lymphocyte depletion with a subsequent decrease activation of endothelial cells following reperfusion. Using an in vivo, skeletal muscle reperfusion model, Chappell et al [47] elegantly demonstrated that pretreatment with ATG reduced microvascular leukocyte adhesion, leukocyte count, and improved blood flow velocity.

A new class of immunosuppressive agents that inhibit T-cell egress from lymph nodes and other lymphoid tissues via the G-protein–coupled receptor, sphingosine-1-phosphate 1 receptor, has recently been developed. FTY720 is a nonselective S1P receptor agonist, the activation of which requires phosphorylation. SEW2871 is a newer agent that is a sphingosine-1-phosphate 1–selective agonist and does not require phosphorylation to activate the receptor. Both novel immunosuppressive agents have been used in IRI models. FTY720 has been protective in a variety of IRI models. Several renal models have demonstrated reduced serologic and histopathologic damage, as well as increased survival after IRI or transplantation in animals treated with FTY720 [48–52]. FTY720 administration has also been shown to reduced post-IRI apoptosis and increased tubular proliferation [51]. FTY720 given daily to rats after IRI had significantly decreased serum creatinine levels and proteinuria 30 days out from injury when compared to untreated rats. The FTY720 treated rats also had significantly reduced interstitial fibrosis scores at 30 days post-IRI. Coupled with this finding was reduced expression of TGF β -1, a potent mediator of fibrosis [53]. This suggests that FTY720 may alter the lymphocytic response to IRI and may decrease the subsequent fibrotic changes by down-regulating TGF β -1. Similar findings were seen in a rat model of renal transplantation [54]. SEW2871 administration is also ameliorates renal IRI by inhibiting T-cell infiltration from both renal and extrarenal lymphoid tissue [55,56]. Lien et al [57] confirmed that SEW2871

pretreatment results in less renal ischemic injury. They also found a marked reduction in the expression of P-selectin, ICAM-1, and TNF- α , suggesting that SEW2871 modulates leukocyte adhesion and the proinflammatory response.

Chemokines regulate the activation, differentiation, and migration of leukocytes by binding to specific surface receptors on target cells. The blocking of certain chemokines and their receptors may alter the lymphocyte response to IRI. CCR5 is the chemokine receptor for RANTES and MIP-1, both of which are involved in leukocyte trafficking. CXCR3 is expressed primarily on activated T cells and natural killer cells, and both CCR5 and CXCR3 are preferentially expressed on T_H1 cells. CCR5 and CXCR3 have been reported to be critical for the recruitment of cells into sites of inflammation following injury [58,59]. By pretreating mice with TAK779, an antagonist targeting both CCR5 and CXCR3, Akahori et al [60] showed a reduced injury phenotype in an intestinal IRI model. There was decreased expression of TNF- α , IFN- γ , and IL-4, suggesting that by altering leukocyte trafficking, TAK779 may modulate the proinflammatory response seen in IRI.

Leukocyte adhesion is required for inflammatory cells to infiltrate damaged tissues. Leukocyte adhesion is mediated by the selectins, which consist of three different trans-membrane receptors, E-selectin, P-selectin, and L-selectin. L-selectin is constitutively expressed on leukocytes, E-selectin is found on endothelial cells, and P-selectin is found on platelets. Selectin blockade has been shown to reduce IRI in a variety of models [61–67]. Using a pig model of both warm and cold renal IRI, Jayle et al [68] found that blocking the selectins with TBC-1269 reduced IRI, cellular inflammation and development of renal fibrosis. The protective effects of TBC-1269 in a liver model of IRI occurred through down-regulation of TNF- α and up-regulation of IL-10 [69]. Other adhesion molecules including ICAM-1, CD11/CD18, and integrins are important in the initiation of IRI [10]. Monoclonal antibody blockade of ICAM-1 confers protection in a rat model of IRI when given before or after the injury [70]. ICAM-1 knockout mice were similarly protected from renal IRI [71]. Blocking CD11/CD18 in rats also conferred protection from renal IRI; it was postulated that the leukocyte adhesion molecule blockade resulted in decreased neutrophil recruitment and infiltration [64]. However, in a model of severe brain IRI, vinblastine induced neutropenia was not protective suggesting that it may be a lymphocyte adhesion molecule interaction that is the early mediator of IRI [72].

Costimulatory signaling is required for T-cell activation. Altering costimulatory molecule signaling may inhibit the T cell response to injury. Several studies have shown the protective effects costimulatory blockade has on IRI models. Blockade of the CD28-B7 pathway with cytotoxic T-lymphocyte antigen-4Ig decreased proteinuria and improved long-term survival in a renal model of IRI [73,74]. However, selective blockade for the B7-1 pathway was not protective, suggesting that the B7-2 may be the mediator of IRI. This likely occurs by inhibiting T cell infiltration and activation [74]. B7-2 blockade has also been shown to improve renal allograft survival in a primate model [75]. Resveratrol is a phenolic compound derived from red wine that is biologically active and acts as an anti-inflammatory and antioxidant agent. In a rat model of IRI, the resveratrol group had less renal dysfunction attributed to an associated decrease in CD86 (B7-2) mRNA and protein expression [76]. Interestingly, de Greef et al [77] found that it was selective blockade of B7-1 not B7-2 that conferred protection from renal IRI. Animals treated with monoclonal antibodies to B7-1 had less morphologic renal injury, markedly reduced T-cell adhesion in the vasa recta, and less T-cell activation. Using CD154 knockout mice and mice treated with anti-CD154 antibodies, Ke et al [78] showed that disruption of the CD154-CD40 costimulatory signal resulted in less serologic and histopathologic damage in a hepatic IRI model. There was also decreased T-cell infiltration, reduced expression of TNF and T_H1 cytokines, and decreased hepatocyte apoptosis. Similar protective effects were found in a stroke model of IRI where CD40 knockout mice and CD154

knockout mice showed reduced stroke volume [79]. In part, this was attributed to decreased leukocyte rolling and adhesion also seen in the knockout mice.

5. B cells in ischemia-reperfusion injury

B cells play an integral role in the adaptive immune response. Upon activation, each individual cell produces and releases thousands of a single specific and unique antibody or B-cell receptor before ultimately undergoing cell death. The antibody aids in immune response by identifying foreign objects and facilitates their removal, destruction, or neutralization. Specific epitopes on foreign antigen are bound by antibody. This binding action alone can prevent deleterious effects of the foreign particle; alternatively, bound antibodies leads to pathogen removal by macrophages and granulocytes. The bound antibody antigen complex also triggers direct pathogen destruction by activation of the complement pathway.

While there has been much effort in discerning the role of the T-cell population in IRI, currently, there has been little work describing the role of the B cell in IRI. While the role of the T cell can be either pathogenic or protective depending on timing of the injury and type of T cell, the role of the B cell has been found to be predominantly pathogenic in all organs systems ranging from the intestine, heart, kidney, and skeletal muscle [80–89]. Various models have been employed to determine the effects of B cells on IRI using mice that are deficient in either the B cells or complement components which interact with B-cell receptor (Table 2). Austen et al [85] used complement receptor–2 knockout mice (CR2^{−/−}) in their IRI model. CR2^{−/−} mice have defective B-1 cells resulting in a deficiency of natural immunoglobulin M (IgM). This deficiency confers protection from IRI. They found that that muscle injury in the CR2^{−/−} mice reconstituted with CM22, a self-reactive IgM secreting B-1 cell clone, had similar injury compared to WT mice reconstituted with saline and compared to CR2^{−/−} mice reconstituted with WT serum. The injury to the CR2^{−/−} mice reconstituted with CM22 was significantly greater than in CR2^{−/−} mice reconstituted with saline and CR2^{−/−} mice reconstituted with a different IgM clone, CM31. The results suggest that CM22 is a mediator of IRI and suggests that B cells are pathogenic in IRI.

Current research suggests that B-cell isotype IgM mediates IRI. The IgM isotype is secreted as a pentamer or hexamer. Its known functions include detection of the A and B antigen on red blood cells and complement activation. The B cell clone, CM22, was found to be the only clone to completely restore intestinal IRI in RAG 1^{−/−} mice [82]. Sequence analysis of CM22Ig heavy and light chains showed germ line configurations with high homology to V_H sequences from the B-1 repertoire and the V_K of known polyreactive natural IgM. Subsequent work showed CR2-deficient mice had reduced IRI in the intestine that was easily reversed with prepared IgM [90,91]. Zhang et al [87] have shown that natural IgM is implicated in myocardial IRI injury as well.

Using mice deficient in C3, C4, or IgG, Williams et al [92] were able to show that the mechanism of action involves the activation of the complement cascade by the classical pathway in the intestine. Austen et al [85] have seen similar results in the hindlimb. The classical complement pathway begins with the activation of C1 by an antigen antibody complex. The complex involves either IgM or multiple IgG molecules. A cascade leads to the generation of a C1 complex containing various subtypes of C1. This molecule in turn cleaves C4 and C2 and eventually generating the C3 convertase and ultimately the C5 convertase and formation of the membrane attack complex. In the process, various split products are produced. Products such as C3a, C4a, and C5a are anaphylatoxins. They trigger degranulation of mast cells and basophils and capillary permeability. C3a and C5a are involved in chemotaxis of leukocytes. The infiltration of a site with leukocytes and their subsequent degranulation can be pathogenic when complement is activated at inappropriate time, leading to tissue and organ

death. In addition, at least in cardiac tissue and skeletal muscle, the lectin pathway is also implicated in the pathogenesis of IRI [88,93]. The lectin pathway, or mannan-binding lectin pathway (MBL), is another mechanism of complement activation. Where the classical pathway involves C1, the lectin pathway uses MBL to bind to the carbohydrate sugar residue mannose often found on pathogen cell surfaces. Again, a cascade results in the cleavage of C2 and C4 to form the C3 convertase on the cell surface, ultimately leading to the formation of the C5 convertase and the membrane attack complex. Much as in classical complement activation, pathogenesis occurs through inappropriate activation and the degranulation of recruited leukocytes. In skeletal muscle, the two pathways appear to cause differing pathology. Chan et al [86] have found that isolated blockade of the classical pathway alone is protective against vascular permeability and remote pulmonary injury but not histologic muscle injury. However, blocking the lectin pathway alone protects against histological injury but not vascular permeability or remote lung injury. Activation of both the classical complement pathway and the MBL pathway are required for the full spectrum of disease associated with IRI.

At this stage, the future of research in the B-cell response to IRI appears to be the manipulation of the response itself. An intravenous injection of a ligand to block the natural IgM and reduce complement activation would be ideal. Initial work in this regard has been promising. Chan et al [93] have been able to bind the IgM receptor with engineered peptides and block IRI in a hind limb model. The P8 peptide clone, which most avidly bound to the pathogenic IgM, and its 2 natural homologues, N2 and GP1, were used. They found significant attenuation of skeletal muscle injury in those animals treated with P8, N2, and GP1, compared to animals receiving saline or the same mass of a random peptide. The level of protection from injury is comparable to that seen in the absence of antibody altogether.

6. T cells in the healing response from IRI

Although most evidence has linked lymphocytes with the early tissue injury response after IRI, emerging data has associated lymphocytes with healing from IRI. van Weel et al [94] studied the role of CD4⁺ lymphocytes in a murine model of hind limb ischemia. Femoral artery occlusion was performed in mice that had been depleted of CD4⁺ cells using anti-CD4 antibodies and in control mice without depletion. Collateral vessel formation, perfusion recovery, and rate of perfusion recovery were all impaired in CD4 depleted mice compared to control mice at 7 days. Femoral artery occlusion was also performed in major histocompatibility complex class II-deficient mice that lack mature peripheral CD4⁺ T cells. When compared to control mice, major histocompatibility complex class II-deficient mice had significantly decreased paw perfusion recovery during the 4 week observation period following IRI. Stabile et al [95] also confirmed the importance of CD4⁺ cells in the angiogenic response to acute hind limb ischemia. Using CD4^{-/-} mice, they found delayed recovery of hind limb function and increased muscle atrophy and fibrosis in the knockout mice compared to WT. The mechanism by which CD4⁺ T cells may mediate collateral vessel development was interpreted to be due to macrophage recruitment and augmentation of vascular endothelial cell growth factor because both were diminished in the CD4^{-/-} model.

Stabile et al also examined how CD8⁺ cells affect angiogenesis following hind limb IRI [96]. They noted reduced blood flow recovery in CD8^{-/-} mice compared with WT mice. The CD8-deficient mice also had increased muscle atrophy and fibrosis compared to the WT. CD8^{-/-} mice had significantly decreased IL-16 expression and CD4⁺ T-cell recruitment at the site of collateral vessel development. Adoptive transfer with CD8⁺ T cells was performed on the CD8^{-/-} mice immediately following IRI. The reconstituted mice showed normalization of IL-16 expression at levels near that of WT mice. There was significant CD4⁺ cell infiltration of the ischemic limb, faster blood flow recovery, and reduced hind limb muscle atrophy and fibrosis in the CD8 reconstituted mice as well.

Regulatory or suppressor T cells have been found to have a variety of functions including modulating the immune system. This occurs through many different mechanisms including inhibiting the activation and proliferation of other T cells, a process likely mediated by IL-10, TGF- β and cytotoxic T-lymphocyte antigen-4. T regulatory cells modulate both the adaptive and the innate immune response. In an islet cell transplant model, cotransfer of regulatory T-cells with islet cells reversed the inflammatory chemokine and chemokine-receptor expression seen in the transfer of cytokine-stimulated islet cells alone [97]. There was a significant leukocytic, neutrophil-rich infiltrate and destruction of islet architecture in cytokine-stimulated grafts, but this was ameliorated when the grafts were co-transferred with CD4⁺CD25⁺ T regulatory cells. Cotransfer with CD4⁺CD25⁺ T regulatory cells enhanced islet cell engraftment [97]. These data would suggest that T regulatory cells reduce the proinflammatory sequelae of IRI by altering the chemokine and cytokine milieu. In a murine model of kidney IRI, depletion of CD4⁺CD25⁺ T regulatory cells with an anti-CD25 monoclonal antibody resulted in higher necrotic indices at 3 days, but not at earlier time points, suggesting that T regulatory cells may play a role in the recovery phase of IRI [98].

7. Conclusion

Lymphocytes contribute to the pathogenesis of IRI. Traditionally thought of as innocent bystanders, there is now a growing body of evidence suggesting a variety of roles for lymphocytes during all phases of IRI. In brain, heart, lung, liver, intestine, and kidney models of IRI, lymphocytes mediate tissue injury and possibly repair as well. These findings suggest the novel immune modulating therapeutics may be beneficial in preventing and treating IRI. Furthermore, altering the postinjury lymphocyte milieu may augment tissue healing.

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Table 1

Indirect evidence of T cells in IRI

Publication, year	Organ/Species	IR Model	Findings
McCarter et al [39], 2001	Brain Rat/Mouse	Warm	FK506 reduced cerebral infarct size in both permanent and transient models of middle cerebral artery occlusion.
Kelly et al [70], 1994	Kidney Rat	Warm	Monoclonal antibody blockade of ICAM-1 reduces histopathologic injury
Akahori et al [60], 2006	Intestine Mouse	Warm	Pretreating mice with chemokine receptor blockade TAK779 reduced injury phenotype; altering leukocyte trafficking resulted in decreased expression of TNF- α , IFN- γ , and IL-4.
Nakao et al [35], 2003	Heart Rat	Transplant	Animals treated with low dose CO had improved long term survival and showed decreased CD3 ⁺ T cell infiltration.
Haanstra et al [75], 2003	Kidney	Transplant	Blockade of the co-stimulatory molecule B-2 improved renal allograft survival
Wang et al [54], 2004	Monkey Kidney Rat	Transplant	FTY720 improved long-term allograft outcomes by reducing lymphocyte infiltration and TGF- β expression
Ke et al [78], 2005	Liver Mouse	Warm	Disrupting the CD154-CD40 costimulatory pathway using monoclonal antibodies or CD154 knockout mice attenuates hepatic IRI
Beiras-Fernandez et al [46], 2006	Skeletal Muscle Monkey	Warm	Thymoglobulin-pretreated animals had less histopathologic damage, less infiltrating leukocytes and less peripheral lymphocytes.
Lien et al [57], 2006	Kidney Mouse	Warm	SEW2871, an inhibitor of T cell egress from lymphoid tissues, ameliorates renal IRI; it also reduces TNF- α , P-selectin and ICAM-1 expression.
Jayle et al [68], 2006	Kidney Pig	Warm / Cold	Selectin blockade with TBC-1269 reduced renal IRI, cellular inflammation, and renal fibrosis
Ibarra et al [30], 2007	Brain Rat	Warm	Cop-1 treated rats showed significantly improved neurologic function and significantly decreased histopathologic damage.

Table 2

B cells in IRI

Publication, year	Organ/Species	IR Model	Findings
Weiser et al [89], 1996	Skeletal Muscle Mouse	Warm	IgG-, C3-, or C4-deficient mice protected from injury; WT serum transfer restores injury.
Burne-Taney et al [83], 2003	Kidney Mouse	Warm	B cell-deficient mice (mu MT) protected from injury; serum transfer partially restored injury, but B-cell transfer alone does not restore injury.
Austen et al [85], 2004	Skeletal Muscle Mouse	Warm	Cr2 gene knockout (KO) mice are protected from injury; B-cell transfer with CM22 and WT serum transfer restores injury.
Zhang et al [82], 2004	Intestine Mouse	Warm	RAG1 KO protected from injury; B-cell transfer with CM22 restores injury.
Chan et al [93], 2006	Skeletal Muscle Mouse	Warm	Binding of preformed IgM clone necessary for IRI; specific peptide blockade of IgM clone attenuates injury.
Zhang et al [87], 2006	Heart Mouse	Warm	Cr2 KO mice protected from injury; WT IgM transfer restored myocardial reperfusion injury.
Busche et al [30], 2007	Heart Mouse	Not Listed	IgM/MBL KO mice reconstituted with IgM/MBL KO plasma had significantly less injury compared to IgM/MBL KO mice reconstituted with WT plasma and WT mice reconstituted with WT plasma