Neutrophils – a key component of ischemia reperfusion injury

Zoe Victoria Schofield[1], Trent Martin Woodruff[2], Reena Halai[1], Mike Chia- Lun Wu[2] and Matthew Allistair Cooper[1a]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia; [2]School of Biomedical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia

[a]Corresponding author - Professor Matthew A. Cooper, Institute for Molecular Bioscience, The University of Queensland, 306 Carmody Rd, PhD, St. Lucia, 4072, Queensland, Australia, Tel: +61 7 3346 2044, Fax: +61 7 3346 2090, Email: m.cooper@uq.edu.au

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Abstract

Ischemia reperfusion injury (IRI) is a common occurrence following myocardial infarction, transplantation, stroke and trauma that can lead to multiple organ failure, which remains the foremost cause of death in critically ill patients. Current therapeutic strategies for IRI are mainly palliative, and there is an urgent requirement for a therapeutic that could prevent or reverse tissue damage caused by IRI. Neutrophils are the primary responders following ischemia and reperfusion and represent important components in the protracted inflammatory response and severity associated with IRI. Experimental studies demonstrate

neutrophil infiltration at the site of ischemia and show that inducing neutropenia can protect organs from ischemia reperfusion injury. In this review, we highlight the mechanisms involved in neutrophil recruitment, activation and adherence and how this contributes to disease severity in IRI. Inhibiting neutrophil mobilization, tissue recruitment, and ultimately neutrophil-associated activation of local and systemic inflammatory responses may have therapeutic potential in the amelioration of local and remote tissue damage following IRI.

Keywords – Inflammation, MOF, Migration, Cytokines.

Ischemia Reperfusion

Clinical Setting of Ischemia Reperfusion

Ischemia reperfusion (IR) has been recognised as a cause of clinical sequelae for over half a century (1) and remains a comon occurrence in coronary bypass surgery, organ transplantation, gut hypo-perfusion and stroke (2,3). IR is recognised as a complex cascade of events including interactions between vascular endothelium, interstitial compartments, circulating cells and numerous biochemical entities that follow ischemia. Inflammation is a key mediator of IR and aspects of the involvement of the innate immune system has been reviewed by others (3–5). Despite our knowledge of the pathophysiology of IR, injury caused by IR precedes clinical observation, and once apparent, it is often too late for intervention. Therefore, there is still a need for a therapeutic that could prevent or reverse the effects of the injuries caused by IR (2). A number of failed clinical trials demonstrated that intervention during the first seconds of reperfusion is imperative, and thus the window of opportunity during reperfusion is limited. Therefore therapeutic options need to be fast acting, readily available by clinicians and not adversely damaging in their own right.

Causes and Effects of Ischemia Reperfusion Injury

IR is initiated by an ischemic episode, where blood supply is restricted to a portion of an organ or the whole organ, initiating cell death which is further exacerbated when blood flow is returned. Ischemia results in tissue hypoxia that causes a build-up of metabolic intermediates and reactive oxygen species (ROS) namely, superoxide, hydrogen peroxide, and hydroxyl radicals. ROS species increase intracellular calcium, cause pH changes, and concomitantly deplete ATP, resulting in damage to cell organelles and leading to necrotic cell death (2,6). ROS production during short bouts of ischemia can be resolved by free radicals and antioxidants such as nitric oxide (NO).

However, excessive periods of ischemia, ranging from a few minutes to half an hour or more (7–9), depending on the organ, cause irreversible effects which are amplified upon reperfusion. Reperfusion floods the ischemic tissue with oxygen. This activates metabolic intermediates and ROS resulting in an overwhelming inflammatory response causing ischemia reperfusion injury (IRI). Increased ROS quenches the production of NO, damages endothelial cells resulting in loss of barrier integrity and release of ROS into the extracellular matrix (9,10). This increases expression of adhesion molecules (3); acts as a chemoattractant for neutrophils, initiating their recruitment (10); activates the complement cascade (11,12) and promotes apoptotic cell death (13,14). Resident macrophages and damaged endothelial cells release pro-inflammatroy cytokines further recruiting, activating and aiding in migration of neutrophils. This results in an overwhelming inflammatory response that if the body fails to regulate, can lead to acute respiratory distress syndrome (ARDS) and, or systemic inflammatory response syndrome (SIRS) which are central to the pathogenesis of multiple organ failure (MOF) (6,15–17), which has a 70% mortality rate (18). IRI physiology is complex, but indisputably the primary response cells to IRI are neutrophils, which can infiltrate the damaged tissue within minutes of activation. Several studies in the 1980's and 1990's investigated the role of neutrophils in IRI (19) but in the past two decades more emphasis has been given to molecular, rather than cellular, targets such as complement receptors (20), toll like receptors (21), reactive oxygen species (ROS) [20] and the pro inflammatory cytokines such as tumour necrosis factor-alpha TNF-α, which has subsequently been shown to not be involved in IRI (22). The role of cytokines, ROS, complement and toll like receptors cannot be ignored in IRI as they have a major role in the pathogenesis of IRI as they support, activate, recruit and amplify the destructive function of neutrophils. However, recent studies have returned focus to the role of neutrophils as a key player in the pathophysiology of IRI (12,23–25). Therefore we will highlight the interactions these

have with neutrophils and how this creates a feedback loop of neutrophil recruitment and excessive damage at the site of IR and how this can result in MOF.

Neutrophils in Ischemia Reperfusion Injury

At the site of IR activated neutrophils further exacerbate host tissue damage through release of ROS, proteinases and cationic peptides (26). Neutrophils produce a large quantity of ROS when nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase is activated upon adhesion or by pro-inflammatory cytokines (41). Neutrophils block capillaries preventing reperfusion of the tissue, which leads to tissue necrosis and an exacerbated immune response. Neutrophils secrete pro-inflammatory cytokines and chemokines to create a positive feedback loop of neutrophil recruitment and activation (12,28), as illustrated in Figure 1. Furthermore, neutrophil migration causes loss of epithelial barrier integrity and down regulation of junctional adhesion molecule (JAMC). JAMC prevents reverse migration of neutrophils (29), which is associated with ARDS, SIRS and MOF (15,22,26). Ischemia reperfusion can affect every part of the body and is initiated by various mechanisms depending on the organ or area involved. Therefore the overactive state of neutrophils in response to excessive ROS, which is also present in normal tissues at lower levels, rather than activation induced via cytokine signalling , could be one reason why a therapeutic to treat or prevent IRI remains elusive.

We will therefore explore the role of neutrophils in specific organs, the mechanisms involved in IRI in that organ and how neutrophils contribute to disease severity regardless of the mechanisms involved in recruiting and activating them.

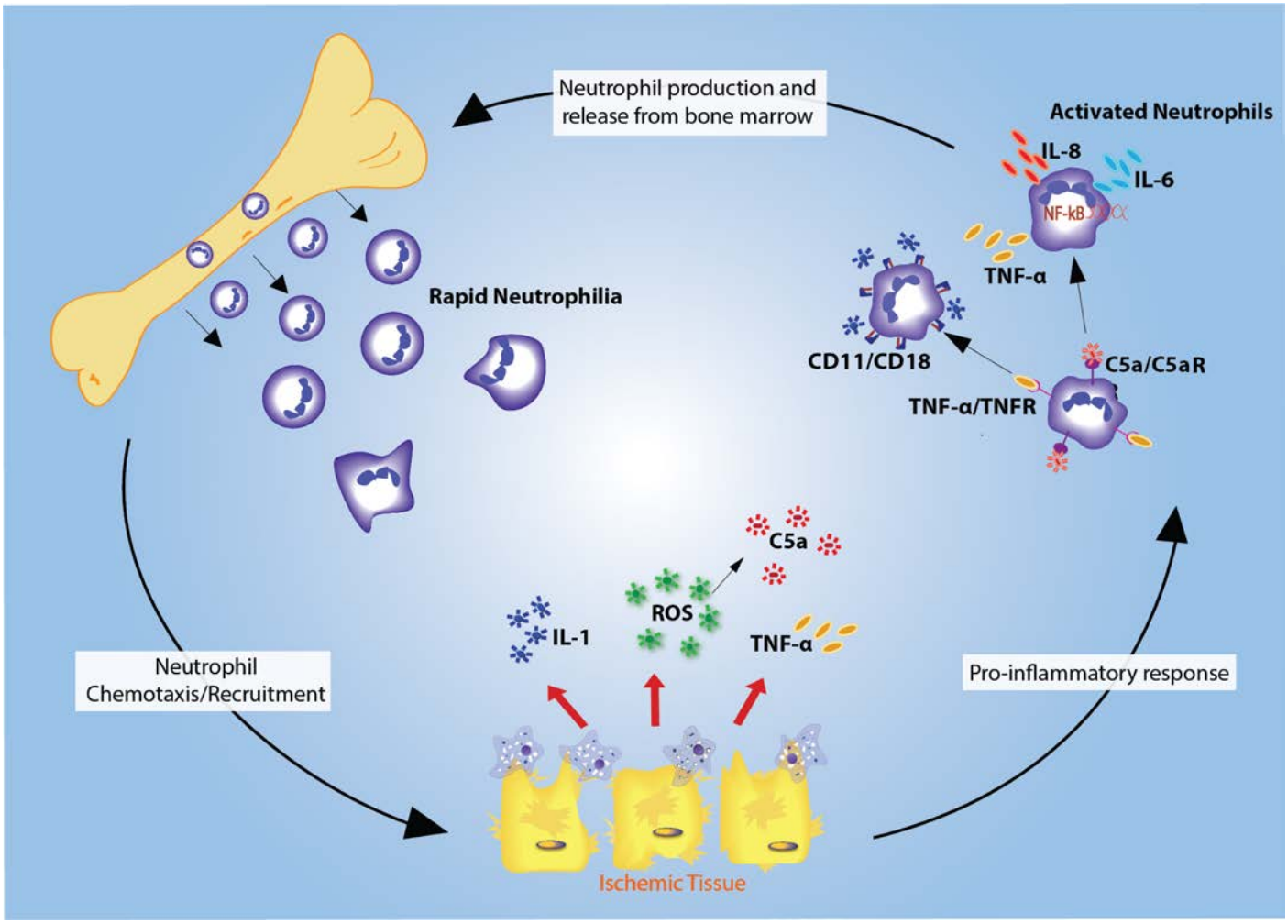

Figure 1: Positive feedback loop of cytokine release and neutrophil recruitment. Ischemic tissue and resident macrophages at the site of ischemia release reactive oxygen species (ROS) and cytokines. ROS activates complement and drives chemotaxis of neutrophils into the ischemic tissue, along with IL-1 and C5a which initiate rapid neutrophilia. Complement proteins and cytokines bind to activated neutrophils at the site of ischemia. This promotes production of further pro-inflammatory cytokines and up-regulates expression of adhesion molecules. C5a binds to the C5a receptor (C5aR) on neutrophils and stimulates NFκB which initiates transcription of TNF-α, IL-8 and IL-6. TNF-α promotes production of IL-1 and up-regulates expression of CD11/CD18 integrins, which are required for firm adhesion to the epithelial/endothelial cell, enabling migration across the endothelial/epithelial barrier. IL-8 promotes neutrophilia and IL-6 stimulates granulopoiesis in the bone marrow. This overwhelming response of neutrophil infiltration and cytokine production overrides protective mechanisms leading to a positive feedback loop of neutrophil mobilization, production, recruitment, migration and subsequently excessive damage beyond that of the initial insult.

Neutrophils in organ specific IR injuries

Heart

Cardiac IR is common after coronary bypass surgery with myocardial infarction being the leading cause of mortality and morbidity in adults in developed and developing nations (30). After

prolonged ischemia restoration of blood flow induces ROS and production of TNF-α, IL-1, IL-6, IL-8, peptide activating factor (PAF) and macrophage inflammatory factor 2 (MIP-2) by endothelial cells, mast cells and myocytes (31). It also activates complement initiating production of C5a (20). These events significantly increase neutrophil infiltration at the site of IRI which directly correlates to infarct size (31). Adhesion molecules, such as CD11, CD18, P-selectin and ICAM-1 on the endothelium are also upregulated which activate neutrophils and enable migration through the endothelium. Neutrophils have deleterious effects in three ways. Firstly they release a large amount of ROS which exacerbates tissue damage (10). This was verified in a dog model, by electron paramagnetic resonance spectroscopy which showed neutrophils as the major source of ROS during reperfusion (32). Secondly, they contribute to the no-reflow phenomenon. This can expand the ischemic insult to over 50% of the capillaries exacerbating tissue damage and necrosis and thus upregulating pro-inflammatory signals, adhesion molecules and neutrophil infiltration (31,33), through the neutrophil feedback loop (Figure 1). Finally, enthusiastic migration of neutrophils across the endothelial barrier leads to tight junction loss (31,34) and potentially MOF.

Various animal models inducing neutropenia in feline, canine, bovine and rodents have exhibited reduced tissue necrosis and myocardial injury (35,36), as well as demonstrating preservation of endothelial function (37). Chandrasekhar and colleagues investigated the role of the pro-inflammatory cytokines IL-6, IL-1β and TNF-α demonstrating that neutrophil depletion in rats significantly inhibited expression of these cytokines independently of NF-κβ (38). Knockout models of P-selectin (39,40) and ICAM-1 (40,41) further corroborate the damaging role of neutrophils in IRI as myocardial necrosis in mice was attenuated in relation with reduced neutrophil infiltration.

Kidney

IRI is a major cause of acute kidney injury (AKI) which has a mortality rate in critically ill patients around 50% and causes significant comorbidity (3,42,43). Neutrophil infiltration in kidney IRI is seen as early as thirty minutes after reperfusion and is evident in both animal models and patient biopsies (25). Awad and colleagues recently carried out an extensive study on the role of neutrophils in kidney IRI in a murine model that clamps the renal pedicles to induce IRI (43). They showed that neutrophil transmigration into the interstitial compartment is responsible for vascular permeability and damage in the kidney.

IRI causes injury to tubular epithelial cells, endothelial cells and resident dendritic cells (DC). Resident DC's produce TNF-α, IL-6, MCP-1, RANTES (44), MIP-2 and keratinocytes-derived chemokine (the mouse analogue of human IL-8)(42), initiating a potent chemotactic gradient for neutrophil recruitment. Interestingly in the kidney IL-8 plays a crucial role in neutrophil recruitment and mediates tissue injury via cytokines, free radical intermediates and proteases (42,44). Increased expression of ICAM-1, P-selectin and IL-8 (45), enables increased adhesion which has been attributed to nephron destruction (46). Upon degranulation neutrophils release proteases, myeloperoxidase (MPO), cytokines and generation of ROS in the outer medulla (42) broadening tissue damage throughout the kidney. Furthermore neutrophils in conjunction with platelets and red blood cells cause blockage to the capillary resulting in the no-reflow phenomenon (25) which amplifies the inflammatory response and thus neutrophil infiltration. Activation of the complement system, specifically C3, C5a and membrane attack complex (MAC; C5b-9) are also seen in kidney IRI (42,47). MAC deposition stimulates TNF-α and IL-6 and down regulates Crry, a complement inhibitor on the tubular epithelium (42).

Lung

Lung IRI can be initiated by several conditions including lung transplantation, cardiopulmonary disease, trauma, resuscitation, atherosclerosis and pulmonary embolism and remains a significant cause of morbidity and mortality (48). Lung IRI can also be initiated from ischemic insult in other organs such as the intestine. Lung injury after intestinal IR is characterised by increased microvascular permeability, alveolar capillary endothelial cell injury, reduced lung tissue ATP levels and neutrophil infiltration (49).

Production of ROS is immediately induced upon reperfusion, primarily from alveolar macrophages and endothelial cells. NFκB, NADPH-oxidase, iNOS and the pro-inflammatory cytokines IL-8, IL-12, IL-18, TNF-α and PAF are activated. These amplify the expression of ICAM-1, CD18 and P-selectin on the endothelial side of the lung (48). These events begin to impair lung function and recruit neutrophils, which generate additional ROS, IL-8, PAF, TNF-α and MPO. Neutrophils are particularly damaging during this phase as they increase lung permeability and facilitate tissue damage (50). Neutropenia induced in a rat model provides protection from tissue damage corroborating that neutrophils are key in the severity of tissue damage (51). Although IL-8 correlates directly to mortality rate after lung transplantation (48) it predominantly induces chemotaxis in neutrophils (52); indicating that higher mortality rates are most likely due to the damage caused by infiltrating neutrophils rather than IL-8 itself.

Liver

The role of neutrophils in liver IR was well defined by Jaeschke and colleague in the early 90's and showed that neutrophils exacerbate liver damage (53). More recently reviews by Ramaiah and Jaeschke 2007 (24) and Kubes and Mehal 2012 (54), provide compelling evidence for the role of neutrophils in liver IR. MIP-2 and keratinocyte chemoattractant are the main chemoattractants in

the liver along with TNF-α , IL-1β and IL-8 promoting neutrophil accumulation and expression of the CD11/CD18 integrin (24). In the liver neutrophils adhere within sinusoids independently of selectins eliminating the requirement of rolling (54). However, activation and accumulation of neutrophils in the sinusoids do not cause tissue damage to the epithelium, as it does in other organs. Only after migrating across the endothelium and in close proximity to the hepatocytes can neutrophils cause damage by oxidative stress, triggered through interaction with CD11/CD18 integrins, NADPH oxidase and MPO (24,55). Transendothelial neutrophil migration therefore is an important step in liver IR which is controlled by expression of CD11/CD18 and the subsequent binding to ICAM-1 (56,57). This was further corroborated by Jasechke and colleagues in 2012(55), when they identified the role of complement in directly priming neutrophils for ROS formation and activation of CD11b expression. They also showed that complement promotes Kupffer cell induced oxidant stress and injury which indirectly enhances neutrophil responses (55). The role of neutrophils in liver IR is further supported through the protective effects seen in animal models of neutropenia (58,59). Although neutropenia is protective, inducing neutropenia in clinical patients would severely immunocompromise them making them susceptible to many pathogenic diseases. This is why we need to focus on modulating neutrophil behaviour rather than preventing it completely.

Gut

Intestinal ischemia reperfusion has a relatively small incidence rate with only 30,000 cases reported per annum in the USA and has therefore not been given as much attention as other organs. However, intestinal IR is often a secondary event to most critical conditions (60), with severe secondary events being associated with atherosclerosis, obesity, diabetes (12,61) and α-adrenergic agents or digitalics (16).

Recent evidence reveals neutrophils are a key player in the pathophysiology of intestinal IRI (12,23,62), and our histological staining of mouse ileum illustrates neutrophil infiltration and villi destruction (Figure 2). Importantly, neutrophil depletion has shown to protect the intestine from late stage mucosal damage and afford protection to remote organs (63–66).

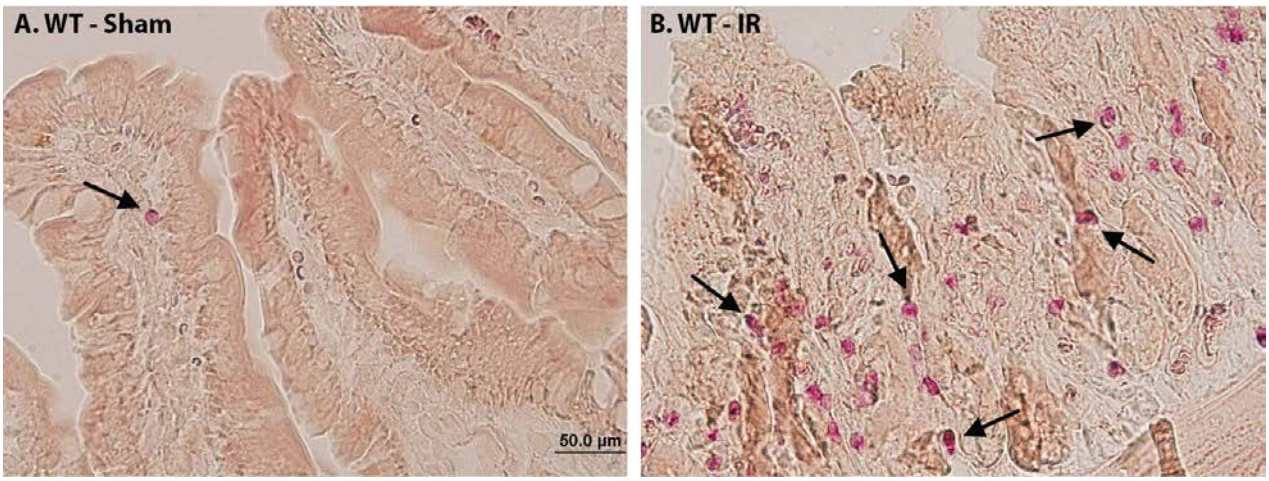

Figure 2: Mouse epithelium showing neutrophil infiltration. Intestinal ischemia reperfusion (IR) increases granulocytic neutrophil infiltration in the intestine, accompanied with destruction of villi (loss of epithelial integrity). Representative sections of ileum from (A) Sham-operated wild-type mice (WT – SHAM) mice and (B) Intestinal IR wild-type mice (WT - IR) showing infiltrating neutrophils (stained red/pink) in the villi of small intestine as indicated by arrows. Granulocytic neutrophils were identified by staining specific leukocyte esterase present predominantly in granulocytic neutrophils. WT-IR mice were subjected to IR surgery in which the superior mesenteric artery was ligated for 30 min and released (reperfused) for 150 min. WT-SHAM mice underwent the same surgery procedures without the artery being ligated. Tissues were collected, PFA fixed and post-processed for the esterase stain (Unpublished data 2013).

The initial insult, as is characteristic with IR in all organs, is from ROS. ROS themselves are key mediators in intestinal IRI; they are a primary source of damage initially compromising the integrity of the endothelial barrier (7,9,67); promote activation of complement; attract neutrophils and enhance expression of cell adhesion markers increasing extravascular migration to the sites of inflammation resulting in vascular injury (12,28,68).

Complement is activated independently through ROS and neutrophil activation and leads to the production of C5a and IL-1β, potent chemoattractants for neutrophils. C5a further stimulates NFκB upregulating transcription of pro-inflammatory cytokines recruiting more neutrophils (7,69). C5aR

knockout models show reduced intestinal mucosal damage, decreased neutrophil infiltration, attenuate neutrophil apoptosis and prevent cytokine release into the plasma (70). TLR2 and TLR4 contribute to the initiation of an inflammatory response (4) as they signal macrophages, monocytes and dendritic cells to further recruit neutrophils through production of cytokines. TNF-α up-regulates expression of CD11/CD18 which forms firm adhesion with ICAM-1 and P-selectin (71,72). In intestinal IRI IL-8, which is secreted from the basolateral surface of the intestinal epithelium, is important for initialising neutrophil migration across the epithelium (73) and neutrophil degranulation (74). Blocking IL-8 in a transgenic mouse model has shown to mitigate intestinal IRI [83]. Platelet levels are increased in parallel to leukocytes in intestinal IRI and bind to neutrophils increasing their adhesive capabilities to the endothelium independently of IL-8. Production of ROS and PAF (76) amplifies neutrophil numbers, pro-inflammatory cytokines and ROS which, fuel tissue damage increase vascular permeability (12).

We recently showed that neutrophil mobilization from bone-marrow, or peripheral pools, following ischemia, plays a key role in inducing intestinal IR injury (23). Importantly, intestinal complement activation was observed after IR, and corresponds with increased circulating neutrophils. Blocking the major complement activation fragment receptor C3aR worsened injury, by increasing the number of mobilized neutrophils in both the circulation and intestine. This intestinal neutrophil infiltration could in turn be blocked by inhibiting the C5a receptor (C5aR), thereby ameliorating intestinal IR pathology. This recent study highlights the importance of the neutrophil and its entry into the blood and subsequently the intestine, in the establishment of intestinal IR injury.

Neutrophils and Multiple Organ Failure (MOF)

IRI in any organ can result in SIRS, ARDS and MOF. In intensive care units 50% of deaths are attributed to MOF (77) and ARDS is fatal in over 40% of patients (26). Neutrophil migration in IRI is

an important part of excessive damage in all organs as highlighted in previous sections and reverse migration has been related to systemic inflammation after remote IR events. Woodfin and colleagues demonstrated this event in a mouse model that initiates IRI in the cremaster muscle or lower limb. Using 3D and 4D imaging technology they observed down regulation of JAMC, which usually prevents reverse migration, and transendothelial neutrophil migration (78) which has been depicted in Figure 3. Neutrophils that undergo reverse migration exhibit enhanced ROS generation and more resistance to apoptosis contributing to systemic inflammation and secondary organ damage (78). Further support for the role of these neutrophils in MOF is a clinical trial analysing the role of neutrophils in the circulatory and lymphatic system of mesenteric IRI. Disruption of the tight junctions increased vascular permeability. This enabled neutrophils and enteric bacteria to translocate into the circulatory and lymphatic system, Neutrophils now primed for enhanced ROS production damage in remote organs, which is highly attributed to ARDS and MOF (15). These findings further substantiate the need for a therapeutic that can reduce the excessive inflammatory response caused by IRI and show how critical neutrophils are in IRI.

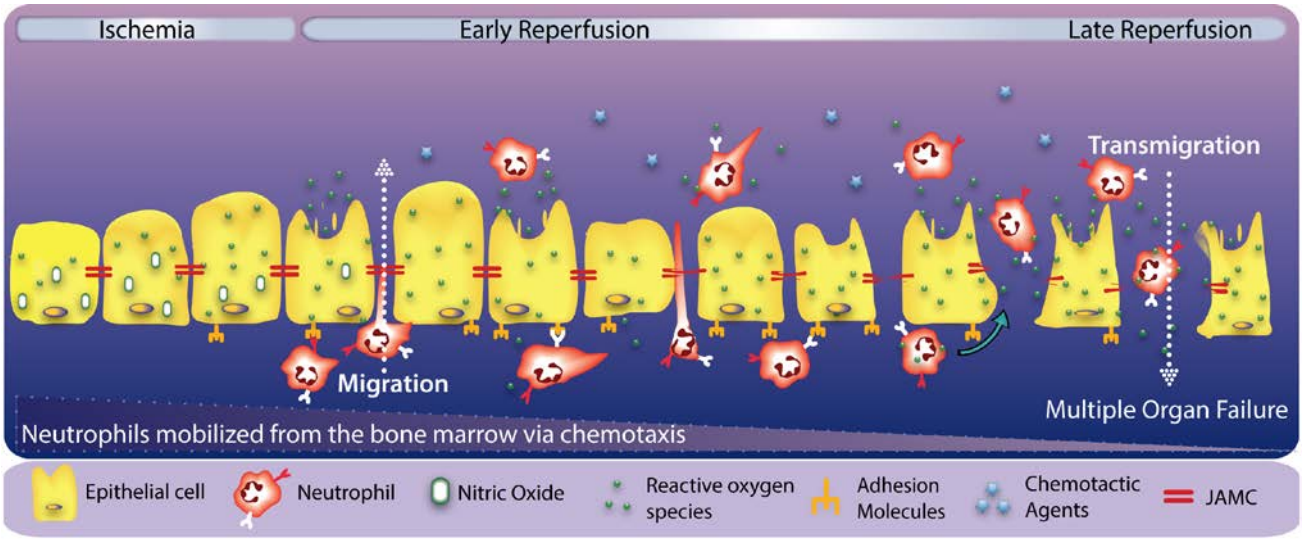

Figure 3: Neutrophil migration is initiated by various chemotactic agents produced at the site of IR. Neutrophils produce ROS and inadvertently destroy local endothelial or epithelial cells that were unaffected by the initial IR insult. Neutrophils become more resilient to apotosis and gain enhanced ROS production. JAMC is disrupted by neutrophil proteases and cell disruption enabling neutrophils to migrate out of the tissue, essentially reverse migration. It can now migrate to other organs and destroy tissues through ROS production leading to ARDS, SIRS or MOF.

Neutrophil targeting therapeutics to treat IRI

To date most therapeutics have targeted cytokines, complement, free radicals, platelet aggregating factor and adhesion molecules in an attempt to resolve the adverse effects of IRI. So far, such agents have been relatively ineffective clinically (7,60,69). Current therapeutic options for IR are merely palliative, offering some relief to the patients discomfort, but failing to improve the underlying condition (79).

One trialled treatment for IRI has been to increase the levels of NO prior to surgery, as many animal studies supplemented with antioxidants demonstrated reduced IRI. During an ischemic state, production of NO is shut down. Upon reperfusion, the ischemic tissue is overloaded with superoxides that quench any remaining NO and produce highly toxic peroxynitrite. Production of superoxides in IRI eventually lead to inactivation of NO altogether (12). Unfortunately, increasing levels of NO in tissue prior to ischemia exacerbated IRI (2). Alternate antioxidant treatments such as Allopurinol, Superoxide dismutase (SOD), iron chelators, N-acetyl cysteine, ethanol, Captopril and Verapamil have also failed to provide conclusive evidence for clinical end point success in animal and clinical trials (80,81). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a potent free radical scavenger, improved survival and renal function in rats subjected to renal IRI (82) and has had success in a clinical pilot study of acute myocardial infarction (83). Recently, stobadine, a novel synthetic pyridoindole antioxidant, which diminishes lipid peroxidation and protein impairment by free radical scavenging and anti-oxidant activity, has been shown to provide significant protection from IRI in rat kidneys (44). Based on evidence showing Hydrogen Sulphide (H_2S) as a modulator of inflammatory events through interaction with leukocytes (84), Sivarajah and colleagues investigated its role in myocardial IR. They demonstrated that H_2S decreases myocardiocyte apoptosis and ICAM-1 expression and neutrophil infiltration (13).

Initial success has been established in pre-clinical models of IRI for a handful of therapeutics that target neutrophils. A monoclonal antibody targeted against the CD11/CD18 integrin showed promising results in animal models (85–87), but clinical trials failed to show a significant reduction in infarct size (88,89). G protein-coupled receptors (GPCR's) have been very successful for a range of disorders and, account for almost a third of all prescription drugs in current use (90). Evidence to date indicates they may also be successful targets for IRI. G protein-coupled receptor 43 (GPR43), which is highly expressed on neutrophils (91), is a receptor for short chain fatty acids (SCFA's). These have shown to reduce the degree of IRI in rat gut using a model of mesenteric ischemia reperfusion (92). Therefore modulation of neutrophils through GPR43 could be a possible future avenue to modulate neutrophil recruitment to organs following IRI. Another GPCR, adenosine 2A (A_{2A}) receptor, also has protective effects. It reduced infarct size in a pig model of myocardial IR (93) and, inhibited adhesion molecules on endothelial cells and reduced neutrophil numbers in a mouse model of kidney IR (94)

Complement inhibition is another attractive target. Gut, liver, kidney, limb and brain models have revealed the role of complement as a key mediator of post ischemic damage (95,96). Further studies supplement these findings, showing that complement inhibitors such as recombinant sCR1 do reduce IRI in various organs (6,72,97–100). However, complement inhibitors have the drawback that they have to block tissue injury whilst preserving its function to prevent infection and eliminate immune complexes; failure to do this leaves the patient severely immunocompromised and susceptible to infection (101). To date, eculizumab is the only clinically available therapeutic that specifically targets the complement system, and is approved for use in Paroxysmal nocturnal hemoglobinuria (PNH) and atypical haemolytic-uremic syndrome (aHUS) (102). It specifically targets C5, preventing its cleavage into C5a; a potent chemoattractant and C5b which forms MAC. C5a is an

important chemoattractant for neutrophils and therefore blocking C5 could reduce neutrophil infiltration in IRI. Unfortunately pexelizumab, a close analogue to eculizumab that also inhibits cleavage of C5, failed to reduce infarct size in a human myocardial infarction trial (103). In animal models of IR complement depletion significantly reduced neutrophil numbers and decreases lung permeability (104). Hence there is a strong possibility that reduction of IRI when inhibiting complement is actually due to a reduction in neutrophil infiltration, inferring that a therapeutic intervention that targets neutrophils specifically could be the key to preventing IRI. In support of this hypothesis, we recently demonstrated that infusion of C3a agonist peptide to mice reduced neutrophil mobilization after intestinal IR, which resulted in reduced tissue neutrophil infiltration and ameliorated disease pathology (23).

Future trends

Many failures have been observed in an attempt to prevent and treat ischemia reperfusion injury (IRI). These failings could be due to a number of reasons, from a lack of understanding of the pathophysiology to insufficiency of the disease models. A main hurdle in drug development is the translation of the efficacy in animal models to humans. Clinical trials for therapeutics that target inflammatory responses have been particularly fruitless in the treatment of IRI, with promising *in vivo* data in animal models failing to relate clinically. The success of therapeutics could be restricted by the availability of models that can truly reflect *in vivo* biology, which has been highlighted recently in a number of reviews (105–107). Furthermore, current studies generally use or target only one component that impedes activation or migration of neutrophils. Targeting several key factors at the same time could provide better protection from IRI without compromising any one area of the immune system and thus resulting in better patient outcomes.

In reality, the insult from ischemia reperfusion is multifactorial and a therapeutic that targets a single molecular aspect of pathology will most likely continue to be ineffective. As such, therapeutics should aim to target multiple pathways, or indeed whole cells such as the neutrophil, to maximise the impact of reducing the inflammatory response caused by IRI. Ischemia reperfusion can occur in just about every part of the body and has a plethora of aetiologies that are specific to the initial insult, area and organ in which it takes place. Regardless, it is apparent that all mechanisms lead to recruitment and activation of neutrophils, which have been shown to correlate with disease severity. Therefore the ultimate therapeutic or combination of therapeutics would ideally dampen the inflammatory signals that mobilize and recruit neutrophils or regulate neutrophils directly in order to prevent IR developing into IRI.

Conclusion

IRI is common during various traumatic and surgical events and responsible for ARDS and MOF, which causes death in over half of all patients affected. Various strategies have been employed to prevent the adverse effects of IR but the complex pathophysiology of IR continues to evade treatment. The inflammatory response is indubitably a key mediator of IRI. In addition, this review has emphasised the importance of neutrophils as a significant contributor to the progression of IRI. Neutrophils contribute to the severity of IRI by exacerbating ischemia through blockage of capillaries (no-reflow phenomenon); escalating the inflammatory response by releasing cytokines; damaging cells unaffected by ischemia through release of ROS and potentially most significantly, by disrupting the endothelial and epithelial barriers which leads to MOF. Therapeutics have targeted several pathways involved in the pathophysiology of IRI but so far have failed to provide an effective therapy to ameliorate outcomes. This review has highlighted the underlying and necessary role of neutrophils in IRI. Further understanding of the mechanisms involved in mobilization,

transmigration and activation of neutrophils in IRI, could lead to a potential therapeutic target that can prevent the onset of IRI.

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