Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury

Jakob Vinten-Johansen*

Division of Cardiothoracic Surgery and Department of Physiology, Emory University School of Medicine, Atlanta, GA 30308-2225, USA

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Abstract

Neutrophils respond to myocardial ischemia–reperfusion in a manner similar to the bacterial invasion of a host. The inflammatory-like response that follows the onset of reperfusion involves intense interactions with the coronary vascular endothelium, arterial wall, and cardiomyocytes in a very well-choreographed manner. Neutrophils have been implicated as primary and secondary mediators of lethal injury after reperfusion to coronary vascular endothelium and cardiomyocytes. The involvement of neutrophils in the pathogenesis of lethal myocardial injury has been inferred from (1) their presence and accumulation in reperfused myocardium in temporal agreement with injury induced, (2) the armamentarium of toxic agents such as oxidants and proteases that are released by neutrophils in reperfused myocardium, (3) responsivity to (recruitment by and/or activation by) inflammatory factors released by reperfused myocardium, and (4) inhibition of lethal post-ischemic myocyte or endothelial cell injury by strategies that interdict neutrophil interactions at any number of stages. However, whether neutrophils are directly involved in the pathogenesis of lethal reperfusion injury in the myocardium, are just pedestrian (first) responders to inflammatory signals released after the onset of reperfusion, or are important to an early but not clinically important phase of pathology are still points of controversy. As with the general area of myocardial protection itself, the failure to reproduce the salubrious effects of anti-neutrophil therapeutic strategies and to successfully translate these strategies into clinical practice has not only fueled the debate, but has jeopardized the further pursuit of myocardial protection therapeutics to improve post-ischemic outcomes. This review will describe the molecular responses of neutrophils to ischemia–reperfusion, discuss the cellular and tissue damage inflicted either directly or indirectly by these white cells, and discuss the physiological impact of interdiction of neutrophil-mediated interactions with myocardial cells at various levels on lethal post-ischemic injury. In addition, it will discuss the arguments for and against the involvement of neutrophils in responses to ischemia–reperfusion in experimental models, and the failure to translate experimentally successful therapy into clinical practice.

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1. Introduction

Neutrophils are an important component of the host defense system, in which they are charged with killing invading pathogens (i.e. bacteria) by a paradigm of search, recognition and destroy. In the immunological response, the neutrophils respond to intercellular signals that identify the invader as foreign, thereby initiation a complex and effective response to rid the body of such infection or noxious material. However, in myocardial ischemia–reperfusion, similar signals of “inflammation” are generated by endothelial cells and cardiomyocytes, and the neutrophil responses are directed against self. What follows is a case of “friendly fire” which may ultimately injure viable endothelial cells and myocytes. Abundant evidence substantiates a role for neutrophils in the myocardium undergoing ischemia–reperfusion: (1) neutrophils are activated [1] and recruited to ischemic-reperfused myocardium within the time course of cellular (endothelial and myocyte) injury [2–5], (2) neutrophils can interact with the other cells to initiate such injury; (3) neutrophils have the tools (oxidants, proteases) to injure myocardium; and (4) neutrophils are activated by pro-inflammatory signals released by ischemic-
reperfused myocardium. Therefore, it is logical to hypothesize that neutrophils participate in reperfusion injury. Myocardial injury may be reversible or irreversible (lethal). Contractile dysfunction in the absence of infarction is reversible since contractile function will be restored in days to weeks. Lethal reperfusion injury is defined as death to cardiomyocytes which are viable at the end of ischemia. These cells die as a direct consequence of reperfusion, and are salvageable by therapeutics which are introduced at or just before the onset of reperfusion. Whether neutrophils contribute to lethal reperfusion injury is highly controversial, and will be discussed in detail in this review. However, there is little evidence to suggest that neutrophils contribute to reversible reperfusion injury in vivo. Although early studies concluded that neutrophils caused post-ischemic stunning [6,7], subsequent studies by Jeremy and Becker [8] and others [9,10] showed that neutrophil depletion by filters or anti-serum does not prevent stunning after brief coronary artery occlusion, implying that neutrophils are not involved in the pathogenesis of stunning. Therefore, the general consensus is that neutrophils are not involved in reversible myocardial reperfusion injury [11,12]. The discussion below will focus on the role of neutrophils in lethal reperfusion injury.

In addition to the acute response to myocardial ischemia–reperfusion contributing to infarction, neutrophils are also important in the longer-term process of healing of infarcts. The inflammatory response is a vital function in healing and scar formation in a myocardial infarct [13], without which aneurysmal rupture is a likely result. However, the seminal observations by Engler et al. [2–4,14] and Dreyer et al. [15,16] suggest that the acute responses of neutrophils after ischemia–reperfusion are more likely involved in an early pathogenic process rather than in longer-term reparative processes. Since these early reports, information has supported the specific recruitment of neutrophils to ischemic-reperfused myocardium, the release of noxious products by neutrophils that can directly injure myocytes and coronary vascular endothelial cells, and subsequently cause physiological damage. Therefore, neutrophils may be involved in the early events of inflammation during which damage is produced to the ischemic-reperfused area at risk. However, that same inflammatory process is a prerequisite for the healing process to take place.

2. Neutrophil-derived mediators of injury

2.1. Enzymes

Neutrophils release more than 20 different proteolytic enzymes such as acid hydrolases, the serine protease elastase contained in azurophilic granules, and the metalloproteinases collagenase and gelatinase (Fig. 1). Elastase actually catalyzes the breakdown of a number of substrates including types III and IV collagen, immunoglobulins,
complement fragments, fibronectin, and proteoglycans. The primary target of proteolytic enzymes is the extracellular matrix and its elastin, collagen, proteoglycan and glycoprotein constituents. Unlike reactive oxygen species (ROS) which are short-lived and react largely without specificity and thereby cause widespread destruction, granular enzymes catalyze specific reactions, and have relatively long half-lives in tissue.

2.2. Oxygen radicals

Neutrophils are a primary source of ROS (Fig. 1) [17,18], including superoxide anions generated by activity of the multicomponent NADPH oxidase [19]. The superoxide anion thus formed is rapidly broken down to hydrogen peroxide (H$_2$O$_2$) by superoxide dismutase, and then to hydroxyl radical. In addition, the myeloperoxidase (MPO) system, found in azurophilic granules, converts H$_2$O$_2$ to hypochlorous acid (HOCl) in the presence of halides, such as chloride and iodine anions. HOCl oxidizes various amines to toxic chloramines. All the aforementioned species of ROS have been associated with or directly cause tissue injury and cell death. ROS generation can be stimulated by a number of factors released in vivo during ischemia–reperfusion, and which interact with specific receptors, notably the complement component C5a, platelet activating factor (PAF), tumor necrosis factor-α (TNFα), and interleukins (IL-6, IL-8), and stimulated in vitro by N-formyl peptides such as N-fMLP. The generation of ROS after pro-inflammatory stimulation accounts for the augmented oxygen consumption during the respiratory burst. The neutrophil can be primed by a first exposure to chemoattractants to give a greater and more rapid respiratory burst in response to a second exposure to pro-inflammatory mediator.

3. Triggers of the neutrophil response to reperfusion

3.1. Cytokines and complement

Neutrophils are activated by a vast array of agents derived from multiple cell types in the myocardium, including endothelium, mast cells, and myocytes (Fig. 1). Complement fragments such as C5a [20,21] and C5a$_{des	ext{-}arg}_2$ and cytokines such as TNFα, IL-1, IL-6, IL-8, Neutrophil Activating Peptide-1 (NAP-1), platelet activating factor (PAF), and macrophage inflammatory protein-2 (MIP-2) act as activating or chemoattractant factors that stimulate neutrophil events in the ischemic-reperfused myocardium. These factors are upregulated [22,23] and released from the ischemic-reperfused myocardium [24–27]. An intracoronary infusion of C5a stimulates neutrophil adherence to vascular endothelium and associated decreases in blood flow. TNFα stimulates neutrophil superoxide anion generation and adhesion [28–30]. The systemic levels of these pro-inflammatory agents have been observed to increase after myocardial infarction [31] and cardiopulmonary bypass [32]. For example, IL-6 is derived from hypoxic myocytes and from ischemic-reperfused myocardium. The amount of cytokine released by hypoxic myocytes is sufficient to stimulate transendothelial migration, superoxide anion generation and to cause irreversible damage to myocytes in vitro [31]. In addition, recent evidence suggests that cytokines such as TNFα can be released by degranulation of mast cells during ischemia–reperfusion [33,34]. Other factors released by mast cells such as histamine, serotonin [35] and PAF [36] participate in the inflammatory response to ischemia–reperfusion directly or by stimulating the release of other cytokines such as IL-6. The concept that ischemic-reperfused myocardium releases soluble pro-inflammatory mediators capable of activating neutrophils is supported by the observations of Dreyer et al. [16] Cardiac lymph sampled during reperfusion stimulated shape changes necessary to cause migration and chemokinesis, increase adherence to endothelium, and to increase expression of CD11b/CD18 on the neutrophil membrane. In addition, there was a correlation between neutrophil activation and presence of infarction. In a subsequent study, Dreyer et al. [37] reported that chemoattractive factors contained in cardiac lymph reached a maximum during the first hour of reperfusion. Other neutrophil chemotactic factors have also been observed in cardiac lymph during reperfusion [38]. Cytokine generation in myocardium is regulated at the transcriptional level by NF-κB, which is activated by cytokines themselves or by ROS (Fig. 1) [39–41]. In agreement with a functional relationship of NF-κB with the pathogenesis of myocardial infarction, binding of NF-κB with a decoy oligodeoxynucleotide inhibited the expression inflammatory genes, and reduced infarct size [42].

3.2. Reactive oxygen species

Reactive oxygen species, including superoxide anions (O$_2^-$) generated by the NADPH oxidase system, hydrogen peroxide (H$_2$O$_2$), and hydroxyl anion (·OH) may be involved in inducing the recruitment and activation of neutrophils by triggering the upregulation of adhesion molecules (i.e. P-selectin and ICAM-1) [43–45] and the release of cytokines and complement [46]. The release of such oxidants may also cause direct injury to endothelium and cardiomyocytes.

3.3. Lipid mediators

Lipid mediators act as triggers of neutrophil activation and adherence. The arachidonic acid metabolite leukotriene B$_4$ (LTB$_4$) is released into ischemic-reperfused myocardium primarily by activated neutrophils. LTB$_4$ is a potent chemoattractant factor for neutrophils. Another arachidonic acid metabolite that activates neutrophils and participates in responses to ischemia–reperfusion is the
potent vasoconstrictor thromboxane A₂ (TxA₂), which is released by both platelets and activated neutrophils. TxA₂ stimulates chemotaxis and diapedesis by upregulating expression of CD18 on neutrophils. The phospholipid platelet activating factor (PAF) is released by platelets, neutrophils, basophils, and monocytes. PAF can also be released by ischemic-reperfused myocardium [47] and by endothelium stimulated by cytokines, hydrogen peroxide or thrombin [48]. PAF is involved in priming, generation of \( \ddot{O}_2 \) in the respiratory burst, degranulation, release of arachidonic acid, and chemotaxis of neutrophils. PAF also increases the expression of complement receptors on the surface of neutrophils and thereby increases sensitivity to complement.

4. Cell–cell interactions

4.1. Neutrophil-endothelial cell interactions

The interaction between neutrophils and the vascular endothelium is a central feature of the inflammatory response in the heart and other organs [49]. Normally, the interaction between neutrophils and vascular endothelium occurs in post-capillary venules. This interaction is relatively easy to visualize with intravital microscopy of quiescent tissue (i.e. mesenteric omentum), but the in situ interactions and their physiological consequences are more difficult to appreciate. Therefore, many studies have used the macro- and micro-arterial and venous vascular preparation to model neutrophil-endothelial cell interactions, which show a robust interaction similar to that observed in venules [50–55]. The adherence of activated neutrophils to endothelial cells and/ or myocytes may be a critical step in pathogenesis of neutrophil-mediated injury [49,56–58], and the physiological outcomes of myocardial infarction and apoptosis [55,59–63].

4.1.1. Selectin-mediated interactions

The initial tethering of neutrophils to coronary vascular endothelium is selectin-dependent (Fig. 2). The selectin family of adhesion molecules includes L-selectin (CD62L), E-selectin (CD62E), and P-selectin (GMP-140, CD62P). L-selectin is constitutively expressed on the surface of leukocytes, and is quickly shed after neutrophil activation. Endothelial cells are stimulated to express P- and E-selectin by thrombin, histamine, cytokines (TNFα), and oxygen radicals generated during reperfusion [43,45,64]. E-selectin may not play an important role in the neutrophil-endothelial interactions during early reperfusion [65] since it is not constitutively expressed, and its de novo synthesis occurs 4–6 h after cytokine stimulation, which is in the later phase of integrin-dependent firm adhesion. P-selectin, the predominant selectin involved in neutrophil recruitment during reperfusion, is stored in Weibel-Palade bodies, and is expressed on the surface of vascular endothelial cells within the first few minutes of reperfusion [66], and is stimulated by thrombin, histamine derived from mast cells, and ROS. In the feline

![Fig. 2. Anti-neutrophil therapeutics and the points of action. Some agents like non-steroidal anti-inflammatory (NSAI) agents reduce activation. Others, such as monoclonal antibodies (MABs) and sialyl Lewis, oligosaccharides (SLex Olig) inhibit neutrophil-endothelial cell interactions with the specific adhesion molecules indicated. I–R = ischemia–reperfusion; EC = endothelial cell; SMC = vascular smooth muscle cell; MYO = cardiomyocyte; P = P-selectin; NO = nitric oxide; Other abbreviations are defined in the text.](image-url)
model of ischemia–reperfusion, expression of P-selectin reaches a maximum at 10–20 min of reperfusion, and is subsequently shed as soluble fragments [64,66,67]. Selectins bind to sialylated and fucosylated oligosaccharides, such as sialyl Lewisx. However, the physiological ligand for P-selectin on endothelium is P-selectin glycoprotein ligand-1 (PSGL-1) [68,69], a disulfide link-containing homodimer localized on microvillae of neutrophils. The interaction between P-selectin on endothelium and PSGL-1 on neutrophils results in a loose tethering, or rolling, of the neutrophils along the vascular endothelium, which is a prerequisite for the later stages of firm adherence and transendothelial migration into the extravascular parenchyma [70].

4.1.2. Integrin-mediated interactions

Neutrophil rolling stimulates the upregulation of β2-integrins on their surface, specifically the CD11/CD18 family of adhesion molecules. The β2-integrins consist of a family of heterodimeric membrane glycoproteins with an α (CD11a, b, and c) and a common β (CD189) subunit; activation of the latter is necessary for firm adherence of neutrophils to the vascular endothelium. The three β2-integrin complexes are CD11a/CD18 (LFA), CD11b/CD18 (MAC-1), and CD11c/CD18 (p150,95). CD11a/CD18 is constitutively expressed on neutrophil membranes, while the CD11b/CD18 glycoprotein complex is stored in secondary granules.

4.1.3. Immunoglobulin adhesion molecules

The physiological counterligand of the CD18 complex on neutrophils is the immunoglobulin intercellular adhesion molecule-1 (ICAM-1) localized on the vascular endothelium. ICAM-1 is constitutively expressed at relatively low levels, but is upregulated by pro-inflammatory mediators such as reactive oxygen species, IL-1 and TNFα [40,71]. ICAM-1 is abundantly expressed in the in vivo coronary vascular endothelium, and its increased expression occurs slower (about 4 h) than that of the rapidly responsive P-selectin. Firm adherence of neutrophils to endothelial cells mediated by CD11/CD18 complexes and ICAM-1 is followed by extravasation through the endothelial barrier into the underlying interstitial space and parenchymal tissues. This migration of neutrophils requires the presence of PECAM-1 located at the endothelial cell junctions [72]; blockade of PECAM-1 will inhibit the inflammatory response in ischemic-reperfused myocardium and reduce infarct size [73].

4.1.4. Neutrophil–myocyte interactions

Cytokines stimulate the upregulation of ICAM-1 on cardiomyocytes, and activated neutrophils adhere to cytokine-(IL-1, IL-6, TNFα) stimulated cardiomyocytes by a CD11b/CD18-ICAM-1 dependent mechanism [74]. Damage to the cardiomyocyte may follow this cell–cell contact by release of cytotoxic oxidants and proteolytic enzymes by the neutrophil [75].

5. Time course of neutrophil events during ischemia and reperfusion

Ischemia without reperfusion is associated with a slow infiltration of neutrophils into the area at risk over 12–24 h, starting with migration from the borders of the evolving infarct, peaking between 2 and 4 days after myocardial infarction [76]. Without reperfusion, neutrophil infiltration is restricted to primarily the border zone of the area at risk myocardium, and few neutrophils are found toward the center of the necrotic zone [77]. However, neutrophil infiltration and accumulation is accelerated and increased with reperfusion after reversible occlusion, with a greater accumulation in the subendocardium compared to the subepicardial myocardium [78]. The initial event in the infiltration and accumulation of neutrophils in the area at risk is likely the early interactions with coronary vascular endothelium. Neutrophil adhesion to coronary vascular endothelium occurs rapidly (i.e. within minutes) after onset of reperfusion [79,80]. This adhesion is paralleled by a progressive decrease in coronary vascular endothelial function. Neutrophil accumulation in the reperfused area at risk is most rapid during the first hour in canine models [15]. Data from Zhao et al. [81] show that the abundant neutrophil accumulation in area at risk myocardium is localized in the intravascular space for the first 6 h of reperfusion, followed thereafter by a shift into the parenchyma as they migrate transendothelially over the ensuing 24 h. This time course roughly parallels the progression of necrosis, and therefore may represent an earlier involvement of neutrophils in the inflammatory response to ischemia than previously thought [3,81–85].

6. Physiological consequences of neutrophil-mediated injury in the heart

6.1. Pathogenesis of necrosis

The numerous studies in which anti-neutrophil agents have reduced infarct size strongly support an active role of inflammatory cells in the pathogenesis of myocardial infarction induced by relatively short periods of ischemia (<90 min). Neutrophils can contribute to the demise of cells in ischemic-reperfused myocardium directly or indirectly by a number of mechanisms: (1) release of reactive oxygen species during the early interactions with coronary endothelium which precedes significant transendothelial migration [86]; (2) release of proteases such as elastase and collagenase; (3) embolization in microvessels leading to no-reflow and secondary ischemia [2,4,87–89]; (4) damage to coronary vascular endothelium and upregulation of adhesion molecules implicated in amplification of neutrophil adhesion and emigration [54,55,90–94]; (5) release of mediators such as IL-8 that amplify further the recruitment of neutrophils to the reperfused myocardium, and which
induce vasoconstriction and platelet activation (i.e. by thromboxane B₂ and leukotriene B₄) involved in infarct development and further neutrophil activation [95]; (6) promotion of the interaction with platelets that may potentiate ischemia–reperfusion injury [96]; (7) extension of infarct size and the degree of apoptosis during later phases of reperfusion [63,81]. There are a number of studies that argue against such an extension of infarct size with continued reperfusion [97,98], making this still an area of future investigation. The extent of infarction has been correlated with an increase in the population of accumulated neutrophils in the infarct zone [63,81], and the increase in neutrophil accumulation parallels the increase in infarct size from 6 to 24 h. The observations, however, do not imply a cause and effect relationship.

6.2. Endothelial damage

The coronary vascular endothelium is a single cell layer front line defense against inflammatory reactions. The endothelium is not simply an inert “cellophane” barrier [99] but is an active participant in preventing neutrophil adherence (via nitric oxide [54,100–102] and adenosine [103,104]) as well as in recruiting neutrophils. Neutrophils can directly cause damage to coronary vascular endothelium [90,94]. Co-incubation of neutrophils with coronary artery segments stimulated by thrombin (which upregulates P-selectin expression) [105] in organ chamber systems causes neutrophils to adherence to the endothelial surface and contraction of the artery related to impaired generation of nitric oxide; there is no contractile response to thrombin in the absence of neutrophils. Neutrophil adherence to thrombin stimulated endothelium attenuates agonist-stimulated vasorelaxation responses to the nitric oxide synthase stimulator acetylcholine, while neutrophils in the absence of thrombin have no effect of endothelium-mediated vasorelaxation responses to acetylcholine [90]. Therefore, neutrophils can directly cause damage to coronary vascular endothelium by adherence-dependent mechanisms involving the early adhesion molecule P-selectin [55]. This endothelial dysfunction is observed in studies using in vivo ischemia–reperfusion with prolonged (infarct-producing) coronary artery occlusion [54,106–111]. Endothelial dysfunction was also observed in the early minutes after the start of reperfusion [61], and has been linked to progressive interaction with and adhesion of neutrophils [59], as well as to the generation of \( \frac{O_2}{O_2} \). Risk factors for cardiovascular disease, such as hypercholesterolemia [112] and diabetes [113–116] exacerbate the endothelial dysfunction after oxidative injury. Endothelial dysfunction has been shown to persist for 48 h, but was nearly fully restored by 72 h [81].

6.3. Microvascular injury

Non-ischemic myocardium rarely contains entrapped neutrophils. However, Engler et al. [4] reported that over half of the capillaries observed in ischemic-reperfused myocardium were not perfused. These non-perfused microvessels in the “no-reflow” zone [117] contained numerous adherent neutrophils. In addition, Engler et al. [4] found a positive correlation between the number of entrapped neutrophils and the frequency of blocked microvessels, suggesting an involvement of entrapped neutrophils in the genesis of no-reflow capillaries. Some studies show that the size of the no-reflow zone can be reduced by anti-neutrophil therapy [118]. Tissue edema in the area at risk myocardium resulting from microvascular injury was prevented by leukocyte-depleted blood perfusion [2]. Other causes of microvascular no-reflow have been suggested, including erythrocyte rouleaux formation, and interstitial edema which increases extravascular compressive forces on the microvasculature. However, erythrocyte entrapment or interstitial edema is unlikely to initiate the no-reflow phenomenon [4].

7. Anti-neutrophil therapy

Various approaches have been used to inhibit the neutrophil component of reperfusion injury, ranging from physically or chemically removing neutrophils from the systemic or coronary circulation, to preventing their adhesion molecule-dependent interactions with endothelial cells. A growing body of data supports the link between these early cell–cell interactions and downstream physiological outcomes such as infarct size and the appearance of apoptosis. Anti-neutrophil therapy has potentially important implications for myocardial protection from ischemia–reperfusion in the catheterization laboratory and in cardiac surgery [119]. The various methods used to inhibit neutrophils at specific points are summarized in Fig. 2.

7.1. Leukocyte depletion

Relative depletion of circulating neutrophils can be achieved by neutrophil-specific filters, anti-serum containing antibodies against neutrophils, and chemical methods. Neutrophil filters have been shown to reduce leukocyte plugging [2], post-ischemic arrhythmias, and myocardial edema. Reperfusion with neutrophil-depleted blood reduced infarct size by 47%, and significantly reduced the size of the no-reflow zone [118]. Using sheep anti-canine neutrophil anti-serum, Jolly et al. [5] reported a reduction of infarct size by 37% after 90 min LCx occlusion. However, there was less infarct reduction (30% reduction that was not statistically significant from control) after 4 h occlusion. In a study by Romson et al. [82] using a canine model, neutrophils were depleted by rabbit anti-dog neutrophil antisera administered before 90 min left circumflex coronary artery occlusion followed by 6 h reperfusion. The antisera depleted canine neutrophils by 77 ± 2%. Infarct size (triphenyltetrazolium chloride and histology) was reduced by 42.7% in association with reduced neutrophil infiltration.
Hatori et al. [120] reported a 50% reduction in infarct size with anti-neutrophil antisera in anesthetized porcine model of 60 min LAD occlusion. De Lorgeril [121] systematically depleted neutrophils by combination chemotherapy (mechlorethamine HCl) and rabbit anti-PMN antisera administered 3–7 days before the experiment. This approach reduced infarct size by 35%, which was also accompanied by a significant reduction in the incidence of ventricular fibrillation. However, there may not be sparing of infarction by leukodepletion with longer periods of coronary artery occlusion (i.e. 3 h), although other manifestations of injury such as myocardial blood flow defects may be avoided by a significant reduction in the area at risk, reduced neutrophil accumulation in the area at risk, reduced neutrophil adherence to post-ischemic coronary artery endothelium, and preserved post-ischemic endothelial function [130]. However, other studies failed to show a reduction in infarct size or vascular injury with sialyl Lewisx analogues acutely [131] or long-term [132]. The reason for the disparity in results is not clear. The consensus from all studies taken together is that blockade of P-selectin or the sialyl Lewisx carbohydrate counterpart after relatively short-term ischemia is effective in attenuating early neutrophil-endothelial cell interactions and subsequent pathology down stream.

Carbohydrate selectin blockers, such as the carbohydrate analogues of sialyl Lewisx, inhibit selectin-dependent adhesion of neutrophils to coronary endothelium. Some studies have reported that these carbohydrate analogues reduce infarct size and coronary endothelial dysfunction both short-term [107,128,129] and long-term. Administration of a recombinant soluble PSGL-1 before reperfusion reduced infarct size, attenuated neutrophil accumulation in the area at risk, reduced neutrophil adherence to post-ischemic coronary artery endothelium, and preserved post-ischemic endothelial function [130]. However, other studies failed to show a reduction in infarct size or vascular injury with sialyl Lewisx analogues acutely [131] or long-term [132]. The reason for the disparity in results is not clear. The consensus from all studies taken together is that blockade of P-selectin or the sialyl Lewisx carbohydrate counterpart after relatively short-term ischemia is effective in attenuating early neutrophil-endothelial cell interactions and subsequent pathology down stream.

7.2. Use of selective antibodies to adhesion molecules

The immunoneutralization of specific adhesion molecules on neutrophils, endothelium and myocytes has provided important data on the importance of neutrophils in the pathogenesis of reperfusion injury. Moreover, these data have established the obligatory nature and impact of early neutrophil events on the down stream pathological processes of necrosis, endothelial dysfunction, microvascular injury, and apoptosis.

7.2.1. Anti-P-selectin and E-selectin therapies

Administration of P-selectin antibodies (such as PB1.3, DREG-200, SLex-OS) administered at or just before the onset of reperfusion has been shown to reduce post-ischemic injury, i.e. infarct size, endothelial dysfunction, and blood flow defects [122–126]. Weyrich et al. [124] reported in a feline model of LAD occlusion–reperfusion that immunoneutralization with the P-selectin antibody PB1.3 reduced infarct size by 60%, in association with a reduction in neutrophil accumulation in the area at risk myocardium, and improved post-ischemic coronary artery endothelial function. The endothelial protection was, in part, a result of direct inhibition of P-selectin-mediated adhesion and subsequent neutrophil activity such as oxidant release [80]. Chen et al. [125] also reported that P-selectin immunoneutralization decreased neutrophil accumulation (MPO and histology), and further showed a reduction of ROS generation in the area at risk myocardium, ostensibly linking ROS production to neutrophil activity, as shown by Duilio et al. [18]. These data suggest that blockade of proximal adhesion events attenuate downstream manifestations of ischemia–reperfusion injury. However, some studies suggest that the cardioprotective effects of P-selectin inhibition are modest, although early activation of neutrophils was prevented [127].

7.2.2. Antibodies to the integrin CD11 and CD18 chains

The ability of an antibody to CD11b (MAB 904) antibody to reduce infarct size was first demonstrated by Simpson et al. [133] in a canine model of 90 min coronary artery occlusion followed by 6 h of reperfusion. A subsequent study [134] reported that this acute reduction in infarct size with the same anti-CD11b antibody administered at reperfusion persisted for 72 h, although the agent was administered multiple times up to 48 h after reperfusion. Ma et al. [135] showed in a feline model of ischemia–reperfusion that a monoclonal antibody to CD18 given just before reperfusion reduced infarct size, limited neutrophil accumulation (MPO activity) in the area at risk, and attenuated coronary vascular endothelial injury.

However, not all studies in which CD18 antibodies to adhesion molecules were administered (largely) at reperfusion demonstrate a reduction in infarct size or other manifestations of injury with anti-CD18 antibody therapy [136]. The etiology of this variability in outcomes to anti-neutrophil therapy was studied by Perez et al. [137] in a canine model of 90 min LCx occlusion followed by 3.5 h of reperfusion. Perez et al. [137] showed that the results in post-ischemic tissue are highly dependent on the specific antibody used. Positive results were observed with monoclonal antibodies that attenuated oxygen radical production by activated neutrophils, and the severity of ischemia is important since not all monoclonal antibodies reduced injury if collateral blood flow was low. Williams et al. [138] showed that the duration of ischemia is important since anti-CD18 antibody therapy was effective after 30 min of coronary artery occlusion, but not after 45 min of occlusion. This limited window of efficacy of anti-neutrophil therapy has been observed in studies using inhibitors of adhesion molecules [139,140], and is further commented on later.
7.2.3. Anti-ICAM-1 antibodies

Immunoneutralization of ICAM-1 after onset of reperfusion has been associated with a reduction in infarct size [135,141–144], attenuation of coronary endothelial dysfunction, and reduced microvascular reperfusion blood flow defects [135,141]. Again, reduction of neutrophil accumulation was associated with infarct size reduction in a number of these studies [135,142–144]. Since CD18 is also expressed on myocytes, anti-CD18 antibodies may attenuate direct injury to myocytes by inhibiting neutrophil–myocyte interactions [74]. However, since neutrophils do not migrate into the extravascular compartment for several hours, this is not a likely mechanism in acute studies (≤ 4 h reperfusion), but may be an additional mechanism in longer-term reperfusion studies.

7.2.4. Inhibition of PECAM-1

PECAM-1 is a member of an immunoglobulin superfamily that is expressed constitutively on both neutrophils and endothelium [145]. PECAM-1 is likely involved in neutrophil transendothelial emigration into parenchyma since antibodies to PECAM-1 inhibit migration and accumulation of neutrophils in the extravascular compartment [146]. The involvement of PECAM-1 dependent neutrophil migration and accumulation in the area at risk in the pathogenesis of infarction was demonstrated by Gumina et al. [73].

7.3. Anti-inflammatory agents

It is well-known that steroidal anti-inflammatory agents inhibit neutrophil activation [147] and neutrophil-related post-ischemic injury [148]. Non-steroidal anti-inflammatory agents such as the prostaglandin prostacyclin and various analogues such as taprostene have also been reported to reduce infarct size by inhibition of neutrophil activation [84,149,150]. In early studies, Simpson et al. [149] showed that prostacyclin (PGI2) reduced infarct size, while the purported stable PGI2 analogue SC39902 did not reduce infarct size. The reduction in infarct size with prostacyclin was associated with an attenuation of superoxide anion production by zymosan-activated neutrophils, and a reduction in neutrophil ingress into the infarcted myocardium. Further support for a neutrophil-related mechanism of anti-inflammatory therapy is provided by a study by Curtis et al. [151], in which attenuation of neutrophil adhesion by the anti-inflammatory agent NPC 15669 reduced infarct size by 51%. Other non-steroidal anti-inflammatory agents such as ibuprofen [152,153] and the inhibitors of cyclo-oxygenase and lipoxygenase, BW-755C [84] or nafazatrom, reduce infarct size in association with attenuated neutrophil events. Among negative studies, the prototype study is the clinical trial of high-dose methylprednisolone treatment in patients with acute myocardial infarction [154] in which aneurysmal formation and rupture in some patients were reported. This trial highlighted the double-edged sword of anti-inflammatory therapy, in that long-term treatment can attenuate the healing process as well as the acute inflammatory response in post-myocardial infarction. However, a number of experimental studies have also reported a lack of reduction of infarct size with anti-inflammatory therapy [155,156]. In the study by Reimer et al. [156], the duration of coronary artery occlusion in the anesthetized canine model was 3 h followed by 3 days of reperfusion (coronary occlusion was permanent in the conscious model). Neither ibuprofen nor verapamil treatment was effective in reducing infarct size. However, three hours of coronary artery occlusion may be well beyond the window of salvageability, as suggested by Gumina et al. [157]. The appropriateness and clinical relevance of short-term occlusions versus longer, more clinically relevant coronary occlusions is a critical philosophical conundrum with which investigators must come to terms, and which will be discussed later.

7.4. Adenosine

Adenosine is a cardioprotective autacoid that is present in small quantities (less than 1 μM) in the normal myocardium, and is transiently increased during ischemia by sequential degradation of high-energy phosphates (ATP, ADP, AMP). Adenosine interacts with specific G-protein coupled adenosinergic receptors on the endothelium, myocytes or neutrophils to elicit a wide range of physiological responses. Therefore, adenosine exerts a broad spectrum of cardioprotective effects on key components (neutrophils, cardiomyocytes, endothelium) and compartments (intravascular, interstitial, myocyte) involved during ischemia and particularly during reperfusion [158,159].

The cardioprotection of adenosine has been linked to its potent inhibition of neutrophil functions. Cronstein et al. [160] reported that adenosine inhibited superoxide generation by neutrophils. Later studies determined that this inhibitory effect was mediated by the A2a adenosine receptor [94,161]. However, the A3 adenosine receptor does not seem to directly inhibit neutrophil superoxide anion generation or degranulation, but does attenuate neutrophil adhesion to endothelium in the nanomolar range [162]. The A1 receptor activates neutrophils at low concentrations, which may be overwhelmed by the more potent A2a-mediated inhibition of neutrophils at higher concentrations.

Olafsson et al. [163] first reported that intracoronary adenosine reduced infarct size by 75% and improved regional contractile function 24 h after the start of reflow. Histology demonstrated preservation of endothelial morphology with decreased neutrophil infiltration and plugging in the central necrotic zone. These data strongly suggested a role for inhibition of neutrophils in cardioprotection. Similar results were subsequently reported by others using intravenous administration of adenosine [164] or adenosine receptor-specific analogues [165–168]. The attenuation of endothelial injury with intracoronary adenosine was reinforced by a subsequent study from the same group [169,170]. Jordan et al. [171] used a canine model of 60
min of collateral-deficient (arteriotomy) LAD occlusion with reperfusion achieved via a carotid artery-to-LAD shunt modified to introduce the $A_2a$ receptor-specific analogue CGS-23680 directly into the coronary artery for the first hour of reperfusion. Jordan et al. found that the adenosine $A_2a$-receptor analogue CGS-21680 significantly reduced infarct size, and significantly reduced neutrophil accumulation in the area at risk, and inhibited neutrophil superoxide radical production in vitro and adherence to the endothelium in the area at risk, and inhibited neutrophil superoxide radical production in vitro and adherence to the endothelium of isolated coronary artery segments. Subsequent studies from our laboratory have largely corroborated the beneficial effects of adenosine in models of LAD occlusion followed by both short-term and long-term reperfusion. An adenosine analog, AMP579, that has both $A_1$ and $A_2a$ receptor actions similar to that of adenosine but has a longer half-life, administered at the onset of reperfusion and continued for 2 h post-reperfusion, reduced infarct size, attenuated neutrophil accumulation in parenchymal tissue and adherence to coronary artery endothelium, and preserved endothelial function. These actions of AMP-579 are entirely consistent with the anti-neutrophil effects of adenosine described above.

The intravenous administration of adenosine, however, with its short half-life in blood has yielded mixed results, with some studies showing no benefit [172] while others showed benefit [164,173]. At issue with these variable results is whether adenosine reached the heart in sufficient concentration to exert cardioprotection, and whether a single treatment with adenosine is sufficient to inhibit short-term as well as longer-term pathology. This has obvious implications for the clinical use of adenosine administered by intravenous route, and on the negative outcomes of some clinical trials in which adenosine was administered intravenously at reperfusion. Relevant to this point, Budde et al. [174] recently reported that the failure of adenosine to reduce infarct size and neutrophil accumulation 24 h after reperfusion could be corrected by multiple infusions over the 24-h reperfusion period. Long-term protection with adenosine may require inhibition of neutrophil events (and other events) occurring during both early and late phases of reperfusion. Future clinical trials should focus on intracoronary routes of administration, or on multiple or continuous infusion if given intravenously.

7.5. Protease inhibitors

Neutrophils release serine proteases such as elastase. The serine protease inhibitor, aprotinin (Trasylol®) inhibits neutrophil migration but does not attenuate adhesion to the vascular endothelium [175]. Aprotinin inhibits endothelial cell activation in response to pro-inflammatory stimuli [176,177]. Pruefer et al. reported that aprotinin reduced infarct size and apoptosis potentially be inhibition of neutrophil activities [178]. Protease inhibitors may also attenuate neutrophil activation and recruitment by inhibit-

ing cytokine generation. The serine protease inhibitor FUT-175 reduced IL-6 production by hypoxia-reoxygenated cardiomyocytes [31]. These studies have not demonstrated a direct link to neutrophil involvement in the cardioprotection.

7.6. Local anesthetics

Class I local anesthetics like lidocaine reduce the priming of human neutrophils activated by platelet activating factor, hydroxypatite and G-CSF at a concentration of $10^{-5}$ M [179–182]. Lidocaine also directly inhibits the production [181,183–185] and release [186] of superoxide anions by activated neutrophils, in part by preventing $p47^{phox}$ translocation from the cytosol to the membrane in a dose-dependent manner, ranging from 20 to 200 µg/ml [120,187]. Lidocaine attenuates neutrophil adhesion to vascular endothelium [188], by inhibiting upregulation and expression of CD11b/CD18 on neutrophils. In addition, lidocaine attenuates chemotaxis of neutrophils into vivo inflammatory sites [189,190], most likely by direct concerted actions on activation, chemotaxis, chemotaxis and adhesion. Lidocaine has been suggested to reduce infarct size directly [120,185,191–193], possibly by an inhibitory effect on neutrophils [185–187,192,194–196], although not all studies support this contention [197].

8. Are neutrophils involved in causing or extending lethal myocardial injury during reperfusion?

There is a long-standing controversy over the involvement of neutrophils in lethal reperfusion injury. Reimer et al. [76] raised the question as early as 1989. Since this time, there have been other publications questioning the involvement of neutrophils in lethal reperfusion injury or in post-ischemic injury generally [12,198]. The reduction in lethal post-ischemic injury by inhibition of neutrophils by interventions introduced just before reperfusion has provided the strongest basic science evidence linking neutrophils to the etiology of lethal reperfusion injury. However, as will be discussed in some detail, clinical evidence to confirm this involvement in humans has been disappointingly lacking.

8.1. Evidence against the involvement of neutrophils in reperfusion injury

There are a number of valid arguments marshaled against significant involvement of neutrophils in the etiology of reperfusion injury [199,200]. The discussion below comments on current arguments raised challenging the role of neutrophils in myocardial ischemia–reperfusion injury. Each is a valid argument that asks serious questions regarding the role of neutrophils in lethal injury, particularly on the clinically relevant injury in the setting of acute
myocardial infarction. The surgical data are not discussed in detail.

(1) Reperfusion injury is present in neutrophil-free systems, such as isolated heart preparations. It is well known that post-ischemic myocardium releases cytotoxic substances during ischemia and reperfusion derived from non-neutrophil sources. Endothelial cells in culture release superoxide anion in response to hypoxia-reoxygenation [201] derived from activity of xanthine oxidase [202–204] or NAD(P)H oxidase [205]. Perfusion with buffer solutions in the absence of neutrophils is associated with a small respiratory burst of superoxide anions during ischemia [206,207], or more profoundly during the first minutes of reperfusion [207]. Kevin et al. [206] quantified superoxide anion production in an isolated perfused guinea pig model by dihydroethidium (DHE) fluorescence on the surface of the ventricular wall during ischemia and reperfusion. Superoxide anion generation (DHE fluorescence) increased during ischemia, but increased markedly during early reperfusion, and showed a sustained albeit lower elevated oxygen radical generation during later reperfusion. This timing is similar to that observed in vivo using spin traps and electron spin resonance technology, in which the major superoxide anion burst was observed at early reperfusion, and was attributed largely to the burst of NADPH oxidase activity in neutrophils [18]. Oxidants can stimulate the release of cytokines, complement and other agents [208] from myocardium and endothelium that cause direct injury. The addition of neutrophils to reperfusion buffers causes an incremental release of oxidants [207], promotes adherence [162], and exacerbates systolic and diastolic contractile dysfunction [162,207] over and above non-neutrophil enhanced buffer perfused hearts. Hence, post-ischemic injury in neutrophil-free systems may be related to the multiplicity of cell sources of cytotoxic species.

(2) Neutrophil inhibition does not consistently attenuate lethal reperfusion injury. There are a number of reasons why there is little consensus in animal studies on neutrophil-mediated injury. First, the doses and concentrations of neutrophil-inhibiting drugs vary widely from laboratory to laboratory, and often within a single laboratory. Second, among the greatest concern are the differences between animals and humans. Collateral blood flow in chronic versus acute settings is one issue separating the species. In addition, laboratory studies are performed in otherwise healthy animals, without underlying risk factors such as hyperlipidemia, hypertension, diabetes, and age. The inflammatory responses themselves can differ between humans and animals. These issues will be discussed in more detail later.

(3) Clinical studies using anti-neutrophil therapies have shown negative results. Although clinical studies report evidence of an inflammatory-like response and neutrophil activation in patients with coronary artery disease [209] or unstable angina [1], and interventions such as percutaneous transluminal angioplasty (PTCA) [210,211] or coronary artery bypass surgery [212], clinical trials have generally failed to show salubrious effects with anti-neutrophil therapy [213–215], which is in marked contrast to the generally positive preclinical laboratory studies discussed above. In the multi-center, randomized, double-blind placebo controlled LIMIT-AMI study [214], patients with acute myocardial infarction presenting within 12 h of symptom onset received one of two doses of a recombinant humanized monoclonal antibody to CD18 (rhuMAb CD18) or placebo before commencing PTCA. Patients receiving rhuMAb CD18 demonstrated peripheral leukocytosis at 24 h while there was no change in peripheral neutrophils in patients receiving placebo. In drug-treated patients, there was no significant increase in angiographically determined (TIMI frame count method) blood flow in the culprit coronary artery(ies) or decrease in infarct size (99mTc-sestamibi single photon emission computerized tomography, SPECT) at ≥ 120 h post-treatment compared to the placebo group. Negative results were also reported for the HALT-MI trial [215] in which patients presenting within 6 h of symptom onset were randomized to receive bolus injections of the antibody to all isoforms of CD11/CD18, Hu23FG (LeuKArrest) or placebo before thrombolytic therapy. The plasma concentration of Hu23FG was sufficient to saturate >80% of CD11/CD18 sites for 12–24 h [216]. In this 420 patient study, there was no significant difference in infarct size even after correcting for variations in collateral blood flow and time to onset of symptoms. Overall mortality was low (1.9%) which makes it difficult to show decreases in mortality with treatment, but there was nevertheless a non-significant trend toward lower mortality in the antibody therapy groups (0.8% and 1.4%) versus placebo (3.3%). Other outcome variables (incidence of re-infarction, adverse events, appearance of congestive heart failure, rehospitalization) were not different among groups. Therefore, these clinical studies did not reflect the successes reported by preclinical trials for anti-adhesion molecule therapy.

There are a number of possible reasons for these negative clinical results. First, other medications may have interfered with, and hence masked, the specific anti-neutrophil effect. For example, unfractionated heparin used during thrombolysis and PTCA binds to MAC-1 and interferes with cytokine activation of neutrophils. In addition, glycoprotein IIb/IIIa inhibitors sometimes used during PTCA and surgery inhibit the MAC-1 receptor. However, these medications are accepted clinical practice, and their exclusion is unethical. Any effect of specific anti-neutrophil therapy would have to be in addition to that exerted by these agents, and suggests a limited degree of neutrophil involvement in clinical outcomes. Second, the mechanisms of neutrophil stimulation are very complex and redundant, involving signaling between cytokines with pleiotropic effects, and synergistic and antagonistic effects. Deactivation of one component, i.e. anti-complement or anti-ICAM-1 therapy, is often accompanied by a counterbalancing activation of another component, resulting in perpetuation of the inflammatory effect.
Therefore, single point interdiction strategies (monotherapy) may be frustrated by this redundant inflammatory mechanism, and by the multiple mechanisms involved in reperfusion injury. In this light, strategies using broad spectrum approaches that target multiple components of the neutrophil-mediated responses to ischemia–reperfusion injury may be more successful. Third, the possibility must be acknowledged that neutrophils may not be involved in the pathophysiology of necrosis and other post-reperfusion pathologies in humans as they have been reported to be in animal models. More likely, the timing of therapy may not coincide with the early involvement of neutrophils, and therefore would be ineffective. Alternatively, the duration of ischemia in clinical studies may exceed the window of salvageability, as demonstrated in some animal models [157]. In the two clinical studies discussed above, the duration of ischemia was < 6 h or 12 h, which far exceeds the ≤ 90 min duration of ischemia imposed in animal models. When ischemia was modestly prolonged in some animal models [138–140] anti-CD18 therapy of deletion of the CD18 gene did not reduce infarct size, supporting the relatively narrow window of therapeutic effect. In addition, some anti-adhesion molecule therapies were shown to be effective only in the presence of collateral blood flow, but were ineffective against collateral-deficient severe ischemia [137]. The failure to reduce injury after more prolonged ischemia may be related to break down of endothelial barrier function which would allow unfettered access of the extra-vascular compartments to neutrophils. In addition, the vast majority of animal models fail to incorporate those risk factors commonly presented clinically that exacerbate endothelial dysfunction such as diabetes, hypercholesterolemia, and hypertension. In the few animal studies with such risk factors, inhibition of neutrophil adhesion failed to reduce infarct size, in contrast to success reported in those same models without superimposed risk factors [217, 218]. Therefore, there is concern that animal models may capture a window of therapeutic opportunity with anti-neutrophil therapy that is closed in patients presenting with longer ischemic times and risk factors. More animal studies must be performed using models that more accurately represent the patient with prolonged ischemia and risk factors before the disparity in efficacy in anti-neutrophil therapy between animal studies and clinical studies can be resolved.

9. Concluding remarks

Experimental studies provide strong but somewhat conflicting evidence that neutrophils are involved in the myocardial response leading to lethal injury upon reperfusion. Some anti-neutrophil interventions successfully reducing lethal reperfusion injury reported by some laboratories have not been reproduced by other laboratories using different or even similar animal models. The entry point of neutrophils into the response to reperfusion is at the proximal end of the inflammatory cascade involving very early interactions with the coronary vascular endothelium. This interaction, and other downstream cell–cell interactions, determines in part the more gross physiological outcomes such as endothelial function, microvascular blood flow, necrosis, and apoptosis.

Despite the strong scientific data supporting a role for anti-neutrophil therapy, the translation of this therapeutic potential to the clinical arena has not been realized, and has placed the very credibility of the search for more effective myocardial protective strategies at a crossroads. Even anti-neutrophil strategies with very strong experimental support, such as adenosine and antibodies against the neutrophil CD18 complex, have not shown consistent clinical benefit. This lack of translation into clinical benefit has led to a questioning of the physiological relevance of preclinical studies performed in animal models that do not accurately reproduce the duration and severity of ischemia presented in patients, and fails to take into account the contribution of risk factors such as hyperlipidemia, hypertension and diabetes to the pathogenesis of infarction. Hence, the efficacy of anti-neutrophil therapeutics determined in models of short-term ischemia may be beyond the therapeutic window of clinically relevant coronary artery disease. But the disparity between scientific data and clinical results is likely due also to the diversity of inflammatory mechanisms engaged by the neutrophil, which may render monotherapy or single target approaches ineffective. Hence, combination therapy targeting a broader range of pathways, receptors and the like may be more effective in clinical trials. In addition, multidose or continuous therapy continued over a 24-h period may be required to target both the acute and longer phases of neutrophil events. Future research should focus on the involvement of neutrophils in apoptosis, and longer term reperfusion extending beyond the acute phase of 4–6 h. Moreover, the very basic question of whether the accumulation of neutrophils within an ischemic-reperfused area represents a pedestrian response to injury, or is an active process contributing to injury at the physiological level warrants further investigation. Finally, future investigations should focus on clinically relevant durations of ischemia (6–12 h). A window of myocardial salvage by anti-neutrophil therapy that closes before this time may make the therapeutic strategy as well as the target largely irrelevant, and may be the basis for the failure of clinical trials to recapitulate the successes of basic science studies. In addition, the animal models should incorporate risk factors such as hypercholesterolemia, diabetes, and hypertension to foster translation to the human arena.

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References

[38] Birdsall HH, Green DM, Trial J, et al. Complement C5a, TGF-β1, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the first one to five hours after reperfusion. Circulation 1997;95:684–92.
[40] Fan H, Sun B, Gu Q, et al. Oxygen radicals trigger activation of NF-


Birnbaum Y, Patterson M, Kloner RA. The effect of CY1503, a

Hayward R, Campbell B, Shin YK, et al. Recombinant soluble


Gill EA, Kong Y, Horwitz LD. An oligosaccharide sialyl-Lewisx


Jerome SN, Dore M, Paulson JC, et al. P-selectin and ICAM-1-

Yamazaki T, Seko Y, Tamatani T, et al. Expression of intercellular

Ma X-L, Lefer DJ, Lefer AM, et al. Coronary endothelial and cardiac

Ioculano M, Squadrito F, Altavilla D, et al. Antibodies against in-

Williams FM, Kus M, Tanda K, et al. Effect of duration of ischaemia

Tanaka M, Brooks SE, Richard VJ, et al. Effect of anti-CD18 anti-

Simpson PJ, Todd RF, Fantone JC, et al. Reduction of experimental

Ma X-L, Tsao PS, Lefer AM. Antibody to CD-18 exerts endothelial

Toombs CF, McGee DS, Johnston WE, et al. Myocardial protective

Olafsson B, Forman MB, Puett DW, et al. Reduction of reperfusion

Jordan JE, Thourani VH, Auchampach JA, et al. A3 adenosine re-

Vinten-Johansen J, Zhao Z-Q, Corvera JS, et al. Adenosine in my-

Reimer KA, Jennings RB, Cobb FR, et al. Animal models for pro-

Allan G, Bhattacherjee P, Brook CD, et al. Myeloperoxidase activity

Romson JL, Hook BG, Rigot VH, et al. The effect of ibuprofen on

Vaporciyan AA, DeLisser HM, Yan HC, Mendiguren II, Thom SR,

Romson, Jordan, and Reimer: Animal models for protective


Vinten-Johansen J, Zhao Z-Q, Corvera JS, et al. Adenosine in myocar-


Toombs, McGee, and Johnston: Myocardial protective effects of adenosine. Infarct size reduction with pretreatment and


Toombs, McGee, and Johnston: Myocardial protective effects of adenosine. Infarct size reduction with pretreatment and


[205] Mohazzab KM, Kaminski PM, Wolin MS. NADH oxidoreductase is
a major source of superoxide anion in bovine coronary artery endo-

[206] Kevin LG, Camara AKS, Riess ML, et al. Ischemic precondition-


[208] Shandelya SM, Kuppusamy P, Herskowitz A, et al. Soluble comple-
ment receptor type 1 inhibits the complement pathway and prevents contractile failure in the postischemic heart. Evidence that comple-
ment activation is required for neutrophil-mediated reperfusion in-

[209] Mazzone A, De Servi S, Ricevuti G. Increased expression of neu-


[214] Baran KW, Nguyen M, McKendall GR, et al. Double-blind, randomized trial of an anti-CD18 antibody in conjunction with recombinant tissue plasminogen activator for acute myocardial infar-
ction. Limitation of myocardial infarction following thromboly-


fusion injury is exacerbated in absence of endothelial cell nitric oxide