The neutrophil as a mediator of myocardial ischemia-reperfusion injury: time to move on

Abstract  Granulocytes, especially neutrophils, are recruited in myocardium during the evolution of acute myocardial infarction. Because the neutrophil reaction is most intense during reperfusion and because these cells are a rich source of toxic oxidant species and proteolytic enzymes, it has become a widely held view that neutrophils are an important mechanism of myocardial injury extension during reperfusion. However, on close examination the evidence underlying this contention is equivocal. The basic experimental situation can be summarised thus. (1) All forms of reperfusion injury (i.e., cytotoxic or lethal cell injury, myocardial stunning, endothelial dysfunction, and reperfusion-induced arrhythmias) can be observed in neutrophil-free conditions. (2) “Anti-neutrophil” interventions (e.g., anti-inflammatory drugs, adenosine, anti-neutrophil antisera, leukocyte filters and inhibitors of the various pathways of neutrophil adhesion) do not consistently prevent reperfusion injury and they certainly do not consistently limit infarct size. (3) The time course of neutrophil accumulation in post-ischaemic myocardium may be different to the time course of injury. (4) Despite more than two decades of research, no double-blind, randomised controlled clinical trial assessing an anti-neutrophil therapy in myocardial infarction has yet reported a positive benefit that is attributable to inhibition of neutrophil recruitment. The evidence weighs against a pivotal role of neutrophils as a causal factor in most forms of ischemia-reperfusion injury. An exception may be microvascular injury and capillary plugging leading to the “no-reflow” phenomenon but even here the evidence suggests that the extent of neutrophil accumulation and microvascular injury is determined by, rather than a cause of, myocyte necrosis.

Key words  Neutrophil – polymorphonuclear leukocyte – ischemia – reperfusion – myocardial infarction – stunning – adhesion molecule – endothelium

Intravascular and tissue accumulation of neutrophils (polymorphonuclear leukocytes) is a characteristic component of the inflammatory response in many forms of tissue injury and infection. The intravascular margination and subsequent tissue infiltration of neutrophils is observed in myocardial ischemia-reperfusion injury. A widely-held view is that the neutrophil response in ischemic-reperfused myocardium is a progenitor of secondary inflammatory damage leading to patterns of injury now commonly termed “reperfusion injuries”. These include lethal or irreversible reperfusion injury that may lead to extension of the infarcted zone, myocardial stunning resulting in prolonged depression of post-ischemic contractile function in myocardium that is not
irreversibly injured, reperfusion-induced arrhythmias, and microvascular endothelial damage associated with the “no-reflow” phenomenon. While a contributory role of neutrophils to these reperfusion injuries may seem biologically plausible, the experimental evidence supporting this injurious role is, I would suggest, contentious or insufficiently reproducible to make a convincing case. Here, I will concentrate particularly on disputing the role of neutrophil-mediated cell injury in myocardial infarction. I shall also consider the role of the neutrophil in myocardial stunning, reperfusion arrhythmias, and in the mediation of the “no-reflow” phenomenon.

**Neutrophil accumulation in myocardial ischemia-reperfusion**

Granulocytic infiltration has long been recognised as a histological hallmark of recent myocardial infarction. The time course of histological changes, including neutrophil accumulation, in unreperfused human myocardial infarction was beautifully documented by Mallory et al. more than 60 years ago (40). The first signs of accumulation of granulocytes, predominantly neutrophils, were observed during the 24 hours after the onset of coronary occlusion. The intensity of the neutrophil infiltrate peaked at around 4 days and then practically completely disappeared by 14 days. Mallory et al. remarked

*The exact function of the polymorphonuclear leucocytes is difficult to explain, ... they produce no definite change in the muscle fibers that can be recognized histologically.*

In experimental infarction, neutrophil accumulation in infarcted myocardium was observed histologically after 12 hours ischemia (34). In another study, 2 hours ischemia followed by 2 hours reperfusion was also associated with histologically detectable neutrophil accumulation (59). Intravascular margination of neutrophils, an early event preceding transmigration and interstitial accumulation, was histologically detectable within the infarcted zone after 4 hours of ischemia in a canine coronary artery occlusion model (57). Interestingly, reperfusion following a much briefer (40 min) period of coronary artery occlusion was found to accelerate the accumulation of neutrophils. Many subsequent experimental studies have contributed to a current view that neutrophils accumulate gradually during ischemia, especially at the peripheral edges of the infarcted area, and that reperfusion of the ischemic myocardium intensifies or accelerates neutrophil accumulation in infarcted myocardium (18, 19, 24, 42).

Thus, in myocardial infarction there is clear evidence of an intense intravascular neutrophil accumulation and tissue infiltration. In ascribing a purpose to the neutrophil response, it would seem reasonable to regard this as part of a concerted inflammatory reaction to necrosis. Key aspects of the molecular pathology of neutrophil chemotaxis have been elucidated in recent years. Activation of the complement system components during ischemia and reperfusion and the rapid upregulation or induction of several endothelium- and myocyte-borne adhesion factors including P-selectin, E-selectin, ICAM-1 and PECAM-1, set the scene for the first steps in neutrophil recruitment, i.e., margination and rolling (22, 26). The evolutionary origins of inflammation would suggest that the neutrophil recruitment is one feature of the coordinated response that promotes tissue healing and scar formation following necrosis. This is really not a point of controversy. More problematic are assertions that the neutrophil response is demonstrably deleterious and that this necessary inflammatory response accounts for major forms of secondary injury during reperfusion.

Controversy about the role of the neutrophil in ischemia-reperfusion injury stems from the notion that gained currency in the mid-1980s that the neutrophil influx, accelerated during reperfusion, might account for the free radical burst associated with the early phase of reperfusion (26, 39, 49). Activated and degranulating neutrophils are known to be a rich source of several toxic reactive oxygen and reactive nitrogen species, including superoxide, hydroxyl radical, hypochlorous acid and peroxynitrite anion, as well as lysosomal proteolytic enzymes. The idea that neutrophil infiltration and reperfusion injury go hand-in-hand has almost become an article of faith in some quarters. However, how good is the evidence to support a pivotal deleterious role of neutrophils in the pathophysiology of ischemia-reperfusion injury?

**Anti-neutrophil interventions and infarct size**

Duration of ischemia is the primary determinant of infarct size. The rate of evolution of infarction may be modified by secondary determinants such as the extent of collateral vessel formation and temperature. In humans and in large collateralised experimental species such as the dog, the rate of advance of the necrotic wavefront may be variable but substantial infarction, tending towards a transmural necrosis will be established within a few hours of coronary artery occlusion (47). In small experimental species relatively devoid of a native collateral circulation, such as the rat and rabbit, transmural myocardial infarction may be established within 30–60 minutes of the onset of coronary artery occlusion (63). The question of whether subsequent reperfusion causes further tissue injury beyond that sustained during ischemia is not definitively answered but in relation to