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Targeting inflammatory pathways in myocardial infarction

Panagiota Christia, MD and Nikolaos G Frangogiannis, MD

The Wilf Family Cardiovascular Research Institute, Department of Medicine (Cardiology), Albert Einstein College of Medicine, Bronx NY

Abstract

Acute cardiomyocyte necrosis in the infarcted heart generates Damage-Associated Molecular Patterns (DAMPs), activating complement and Toll-Like Receptor (TLR)/Interleukin (IL)-1 signaling, and triggering an intense inflammatory reaction. Infiltrating leukocytes clear the infarct from dead cells, while activating reparative pathways that lead to formation of a scar. As the infarct heals the ventricle remodels; the geometric, functional and molecular alterations associated with post-infarction remodeling are driven by the inflammatory cascade and are involved in the development of heart failure. Because unrestrained inflammation in the infarcted heart induces matrix degradation and cardiomyocyte apoptosis, timely suppression of the post-infarction inflammatory reaction may be crucial to protect the myocardium from dilative remodeling and progressive dysfunction. Inhibition and resolution of post-infarction inflammation involves mobilization of inhibitory mononuclear cell subsets and requires activation of endogenous STOP signals. Our manuscript discusses the basic cellular and molecular events involved in initiation, activation and resolution of the post-infarction inflammatory response, focusing on identification of therapeutic targets. The failure of anti-integrin approaches in patients with myocardial infarction and a growing body of experimental evidence suggest that inflammation may not increase ischemic cardiomyocyte death, but accentuates matrix degradation causing dilative remodeling. Given the pathophysiologic complexity of post-infarction remodeling, personalized biomarker-based approaches are needed to target patient subpopulations with dysregulated inflammatory and reparative responses. Inhibition of pro-inflammatory signals (such as IL-1 and Monocyte Chemoattractant Protein-1) may be effective in patients with defective resolution of post-infarction inflammation who exhibit progressive dilative remodeling. In contrast, patients with predominant hypertrophic/fibrotic responses may benefit from anti-TGF strategies.

Keywords

myocardial infarction; inflammation; remodeling; animal models; leukocytes; cytokine

INTRODUCTION

Over the last 40 years, development of early reperfusion strategies and advances in both pharmacological and interventional treatment have significantly reduced mortality in patients with acute myocardial infarction [1]. However, improved survival rates due to better myocardial salvage contributed to an expansion of the pool of patients that develop heart failure. In an investigation evaluating temporal trends in survival and prognosis in patients suffering acute myocardial infarction, a marked decline in 5-year mortality (from 41.1% in the 1970s to 17.3% in the 1990s) was associated with a significant increase in the 5-year

incidence of heart failure (from 27.6% in the 1970s to 31.9% in the 1990s) [2]. The pathogenesis of heart failure following myocardial infarction is linked with the development of cardiac remodeling, a constellation of geometric, morphologic, molecular, and functional changes that involve both the infarcted and the non-infarcted myocardium, result in chamber dilation and ventricular dysfunction, and are associated with adverse outcome [3]. The severity of post-infarction remodeling is dependent, not only on the size of the acute infarct, but also on the qualitative characteristics of the reparative response [4], [5], [6].

Because the adult mammalian heart has negligible regenerative capacity, healing of the infarcted myocardium is dependent on sequential activation of inflammatory and fibrogenic signals. Dying cardiomyocytes release their intracellular contents triggering a potent inflammatory reaction that serves to clear the infarct from dead cells and matrix debris, while setting the stage for infiltration of the infarcted myocardium with reparative mesenchymal cells. In the 1980s and 1990s, experimental evidence derived predominantly from large animal studies suggested that post-infarction inflammation may accentuate ischemic myocardial injury in the reperfused heart; thus, inhibition of inflammatory signals was considered a potentially promising therapeutic target [7]. Unfortunately, this promising concept never translated into clinical success, as clinical trials attempting to inhibit inflammation in order to reduce acute cardiomyocyte injury proved unsuccessful. Over the last 15 years, improved understanding of the biology of inflammation has challenged the oversimplified views of the past, leading to identification of both protective and injurious pathways activated by inflammatory signals. Our brief review manuscript discusses the involvement of the inflammatory reaction in injury, repair and remodeling of the infarcted heart. Our goal is not a comprehensive analysis of the cellular events and molecular signals involved in post-infarction inflammation; recent extensive reviews have covered this topic [8], [9]. We rather aim at providing a critical discussion of the basis for the unsuccessful application of anti-inflammatory strategies in myocardial infarction and we identify new therapeutic approaches targeting the inflammatory cascade to protect the infarcted heart from adverse remodeling.

Initiation of the post-infarction inflammatory response

In the infarcted heart, sudden necrosis of a large number of cardiomyocytes results in release of their intracellular contents and initiates an intense inflammatory reaction. Several distinct, but overlapping, pathways play a role in activation of post-infarction inflammation. First, release of subcellular membrane constituents leads to activation of the complement cascade [10]. More than 40 years ago, Hill and Ward demonstrated C3 cleavage in the infarcted myocardium and documented a role for the complement system in leukocyte infiltration [11]. Over the next two decades extensive experimental evidence suggested that complement inhibition consistently attenuates the post-infarction inflammatory response [12], highlighting the critical role of the complement cascade in triggering inflammation in the ischemic myocardium. Second, necrotic cells and damaged extracellular matrix release endogenous alarm signals referred to as 'damage (or danger)-associated molecular patterns' (DAMPs). Hyaluronan fragments, heat shock proteins, High-Mobility Group Box 1 (HMGB1), ATP and mitochondrial DNA may act as key danger signals in the injured myocardium potentially stimulating inflammatory cascades [13], [14], [15], [16]. DAMPs exert their pro-inflammatory actions by stimulating members of the Toll-like receptor (TLR) family. Of the 13 known mammalian TLRs, TLR4 has been identified as a key receptor in mediating the inflammatory response in the infarcted heart [17], [18]. Third, generation of reactive oxygen species (ROS) in ischemic tissue directly induces pro-inflammatory cascades. Marked increases in ROS formation overwhelm the endogenous antioxidant defenses of the myocardium resulting in free radical-mediated cellular injury. In addition, ROS trigger cytokine and chemokine cascades by activating the inflammasome platform in

myocardial cells [19], [20]. Formation of the inflammasome results in generation of active Interleukin (IL)-1, the prototypical pro-inflammatory cytokine that drives expression of inflammatory mediators in the infarct [21]. Fourth, activation of neurohumoral pathways in the infarcted heart (such as catecholamines and the angiotensin/aldosterone axis) may stimulate inflammatory signaling; however, the actions of these mediators extend beyond regulation of inflammation and modulate angiogenic and fibrogenic responses critical to the reparative response.

The cell types responsible for initiating the post-infarction inflammatory reaction remain poorly defined. Clearly, dying cardiomyocytes and degradation products of the cardiac extracellular matrix contribute to the inflammatory reaction by serving as a source of danger signals. Resident cardiac fibroblasts, the most numerous non-cardiomyocytes in the mammalian myocardium, may serve as sentinel cells, sensing ischemia and cardiomyocyte injury and producing inflammatory cytokines and chemokines [22]. Microvascular endothelial cells may also be an important source of inflammatory proteins; endothelial activation and expression of adhesion molecules such as selectins and Intercellular Adhesion Molecule (ICAM)-1 is a crucial step for adhesion and recruitment of leukocytes [23], whereas production of chemokines by endothelial cells in the infarct border zone has been documented in experimental models of myocardial infarction [24], [25]. Another cell type with a potential role in initiation of the post-infarction inflammatory reaction is the cardiac mast cell. Strategically located in the cardiac perivascular space, mast cells contain large amounts of preformed pro-inflammatory mediators (such as histamine and Tumor Necrosis Factor (TNF)- α) and are capable of synthesizing cytokines and growth factors. In the ischemic heart, mast cells rapidly degranulate in response to activation with complement, adenosine, or ROS and release their granular contents, contributing to the initiation of the post-infarction inflammatory response [26].

Leukocyte subpopulations in the infarcted myocardium

Activation of TLR/IL-1 and complement cascades stimulates Nuclear Factor (NF)- κ B signaling, promoting a pro-inflammatory phenotype in endothelial cells and fibroblasts. Activated endothelial cells express adhesion molecules and secrete large amounts of chemokines [27]; immobilization of both CXC and CC chemokines on endothelial and matrix glycosaminoglycans generates a chemotactic gradient leading to recruitment and extravasation of neutrophils and mononuclear cells in the infarcted myocardium.

CXC chemokines (such as IL-8), C5a, leukotrienes and Platelet-activating Factor (PAF) mediate infiltration of the infarct with neutrophils. The migration of neutrophils from the circulating blood to the injured tissue is mediated through activation of a multi-step adhesion cascade [28], [29]. Initially, circulating free-flowing neutrophils are “captured” by the blood vessels and roll along the endothelium of post-capillary venules. This adhesive interaction is mediated by members of the selectin family. L-selectins are expressed in neutrophils whereas P- and E-selectins are expressed in the endothelial surface. The attachment of the neutrophils to the endothelial surface allows them to sense activating mediators (such as CXC chemokines and components of the complement cascade), leading to activation of integrins, adhesion molecules that mediate firm adhesion of neutrophils to the endothelium through interactions with the endothelium-expressed ligand Intercellular Adhesion Molecule (ICAM)-1. Both antibody neutralization studies [30] and genetic loss-of-function models [31] documented the crucial role of β 2 integrins, in recruitment of neutrophils into the infarcted myocardium. Finally, adherent neutrophils transmigrate into the infarct through interactions that may involve endothelial transmembrane proteins, including Platelet Endothelial Cell Adhesion Molecule (PECAM)-1, ICAM-1, vascular-endothelial (VE)-cadherin [29], and members of the junctional adhesion molecule (JAM) family, [32], [33].

Recruitment of neutrophils is followed by infiltration of the infarct with monocytes and lymphocytes [34], [35]. Sequential activation of specific chemokine/chemokine receptor pairs recruits selected mononuclear cell subpopulations in the infarcted myocardium. Early induction of the CC chemokine Monocyte Chemoattractant Protein (MCP)-1/CCL2 mediates early infiltration with pro-inflammatory monocytes that express the chemokine receptor CCR2 and have prominent phagocytic properties [6]. As the infarct heals, inhibitory and reparative monocyte subpopulations are recruited through poorly understood chemotactic mechanisms [34]. However, the phenotype of monocytes in the infarcted heart is dynamically modulated by their cytokine and growth factor-rich environment. Macrophage-Colony Stimulating Factor (M-CSF) is markedly upregulated in the infarct and may drive monocytes to macrophage differentiation [36]. The dynamic environment of the infarct may lead to generation of macrophage subpopulations with distinct functional properties, regulating fibroblast activity, matrix metabolism and angiogenesis and orchestrating the reparative response [37].

Timely resolution of the inflammatory response is required for optimal healing of the infarcted heart

Optimal tissue repair requires timely activation of endogenous inhibitory signals that suppress inflammatory cascades, while activating reparative pathways [9]. Because of the potentially catastrophic consequences of uncontrolled inflammatory activity, multiple STOP signals have evolved for negative regulation of the inflammatory cascade following tissue injury. Because in the myocardium, even subtle structural perturbations result in marked functional impairment, a temporally restrained and spatially restricted inflammatory response is crucial for repair of the infarcted heart. Despite the significance of resolution of inflammation in the injured heart, our understanding of the pathways that negatively regulate the post-inflammatory response remains limited.

All cells involved in cardiac repair likely participate in suppression and resolution of the post-infarction inflammatory reaction. Because of their regulated recruitment and distinct cytokine and growth factor expression profiles, mononuclear cell subpopulations are ideally suited to serve as master regulators of the inflammatory reaction. Our experiments have suggested that monocytes and regulatory T cells (Tregs) expressing the chemokine receptor CCR5 may play an important role in suppression of the inflammatory reaction in healing infarcts [38]; undoubtedly, other inhibitory mononuclear cell subpopulations remain to be identified. Neutrophils may suppress post-infarction inflammation through their death: clearance of apoptotic neutrophils by macrophages triggers release of anti-inflammatory mediators mediating resolution of inflammation. Maturation of the infarct vasculature through acquisition of a pericyte coat may suppress endothelial inflammatory activity providing an additional cellular mechanism for suppression of post-infarction inflammation [39].

Suppression of inflammation may involve a wide variety of inhibitory signals, both secreted proteins (such as Transforming Growth Factor (TGF)- β and IL-10) and intracellular mediators that inhibit pro-inflammatory cascades. Lipid mediators with specialized proresolving functions (such as the resolvins, lipoxins, protectins and maresins) have been implicated in resolution of inflammation [40]; however, their potential role in the infarcted heart remains unknown. IL-10 exhibits a late time course of induction in the infarcted myocardium and is predominantly expressed by subpopulations of macrophages and by T cells [41]. Although IL-10 potently inhibits the production of pro-inflammatory cytokines by mononuclear cells, in vivo studies using knockout animals did not suggest a critical role for IL-10 in resolution of the post-infarction inflammatory reaction [42]. IL-10 upregulation in the infarct may also exert matrix-stabilizing effects by inducing production of tissue inhibitor of metalloproteinases (TIMP)-1 by mononuclear cells [41]. Expression of TGF- β

isoforms is increased in infarcted hearts and may negatively regulate the inflammatory response. Activation of angiotensin II signaling may play an important role in induction of TGF- β in the infarcted heart [43]. TGF- β may exert anti-inflammatory actions by deactivating macrophages, by downregulating pro-inflammatory cytokine, chemokine and adhesion molecule synthesis in endothelial cells, and by promoting differentiation of inhibitory lymphocyte subsets (including Tregs) [44], [45]. In addition to its actions on modulation of inflammation, TGF- β exerts potent matrix-preserving effects and is a key mediator in regulation of matrix remodeling in the infarcted myocardium. TGF- β stimulation induces myofibroblast transdifferentiation and enhances synthesis of both structural and matricellular matrix proteins [46], [47], [48]. TGF- β also suppresses activity of proteases that degrade the extracellular matrix and accentuates synthesis of protease inhibitors, such as TIMP-1 and plasminogen activator inhibitor (PAI-1).

Negative regulation of inflammatory signaling involves activation of distinct pathways in various cell types involved in cardiac repair. Growth-differentiation factor (GDF)-15, a TGF- β -related cytokine, has been identified as essential inhibitory molecule that inhibits neutrophil integrin activation preventing excessive chemokine-mediated leukocyte arrest in the endothelium [49]. Recent experiments from our laboratory have identified Interleukin Receptor Associated Protein (IRAK)-M as an important stimulus for negative regulation of the post-infarction inflammatory reaction [50]; IRAK-M exerts its anti-inflammatory actions by inhibiting TLR/IRAK-1-dependent signaling in macrophages. Fibroblasts also exhibit IRAK-M upregulation; however, fibroblast IRAK-M inhibits the matrix-degrading capacity of the cell without affecting expression of chemokines and cytokines [50]. Moreover, increased expression of cytokine and chemokine decoy receptors by both leukocytes and resident myocardial cells may serve as a molecular sink that terminates inflammatory signaling in the infarcted heart [51].

The post-infarction inflammatory response as a therapeutic target

The concept of inflammatory cardiomyocyte injury—The inflammatory response did not evolve to protect the injured heart. Even in humans where cardiac injury is a common cause of death, myocardial infarction occurs after the reproductive age and does not impose any evolutionary pressure on the inflammatory cascade. Because inflammation evolved to protect injured cutaneous tissues or mucosal surfaces from bacterial contamination, one may hypothesize that the intense inflammation associated with injury may be excessive for the sterile environment of the infarcted heart and detrimental to the delicate structure of the myocardium. The significant injurious potential of inflammatory leukocytes in the myocardium and the extensive neutrophil infiltration observed in the viable border zone of the infarct supported the notion that the post-infarction inflammatory reaction may extend ischemic injury. In the 1980s and 1990s, this hypothesis was tested in large animal models of myocardial infarction. A large amount of experimental evidence demonstrated that antibody neutralization of specific inflammatory mediators (including adhesion molecules, cytokines and chemokines) markedly reduced infarct size following coronary occlusion and reperfusion. Anti-integrin approaches appeared particularly promising, with several independent studies in animal models ranging from rodents to primates suggesting marked reduction in infarct size upon administration of anti-CD11/CD18 antibodies [52], [53], [54], [55], [30], [56] (Table 1). Unfortunately, three small clinical trials showed no effects of early anti-integrin approaches in reducing infarct size in human patients with myocardial infarction [57], [58], [59] (Table 2). Moreover, a large clinical trial targeting the complement cascade failed to produce beneficial effects in patients undergoing percutaneous interventions for acute myocardial infarction [60]. Considering the highly promising results of experimental studies, what is the basis for failure to translate into

the clinical context? Why was there such a gaping disconnect between animal studies and clinical investigations?

First, the enthusiasm generated by the abundance of positive experimental results may have been somewhat misleading. Due to a publication bias against negative experimental studies, published evidence on the beneficial effects of anti-inflammatory strategies may not have adequately reflected the collective experience of the scientific community. More recently, a growing body of evidence, derived from investigations using genetically targeted animals, has challenged the notion that post-infarction inflammation extends ischemic cardiomyocyte injury. P-selectin/ICAM-1 double knockout mice [61], IL-1 type 1 receptor null mice [21] and MCP-1 $-/-$ animals [6] had no reduction in infarct size, despite markedly attenuated inflammatory responses.

Second, animal models have important limitations that prevent assessment of translationally relevant endpoints. Mortality is the most important endpoint in myocardial infarction clinical trials; in contrast, mortality data in animal studies are often difficult to interpret and cannot always be translated into the human clinical condition. Arrhythmias are common causes of death in patients with acute myocardial infarction; however, in mice the small size of the heart and the rapid heart rate may reduce the incidence of arrhythmogenesis. Moreover, mouse models of coronary occlusion do not provide information on the incidence of recurrent coronary events due to plaque instability, a major cause of morbidity and mortality in patients surviving myocardial infarction.

Third, due to the pathophysiologic complexity and heterogeneity of myocardial infarction in human patients, animal models cannot be used to derive direct translational conclusions. Animal models are optimally used to gain mechanistic insights into the role of specific molecular pathways and cell biological responses. For this reason, the goal of a well-designed animal study should be to eliminate variability in order to dissect the role of a specific signal or mediator in the pathophysiologic process of interest. In contrast, in patients with myocardial infarction variations in a remarkable range of factors may affect outcome. Differences in age, gender, genetic background, the pattern of coronary disease, the presence of comorbid conditions (such as diabetes, hypertension and hyperlipidemia), and treatment with various pharmacologic agents have profound effects on the response to myocardial infarction; the complexity of these variations cannot be recapitulated by any animal model. The importance of these factors in altering the biological response to myocardial infarction is illustrated by studies on the effects of aging on the post-infarction inflammatory reaction. Our laboratory has demonstrated that senescent animals exhibit significantly suppressed (and somewhat prolonged) inflammatory activation following myocardial infarction [62]. Because conclusions on the effectiveness of anti-inflammatory approaches following infarction were based exclusively on investigations in young animals (which exhibit very robust and transient inflammatory reactions), the findings may not be translationally relevant to the aged human population presenting with an acute coronary event. Thus, a well-executed animal study should be expected to enhance our knowledge on the mechanisms of disease; this information can then be used to design a therapeutic approach, but does not have direct predictive value regarding success in the clinical context.

Targeting the inflammatory response to protect the heart from adverse remodeling

As the initial enthusiasm for the use of anti-inflammatory strategies to limit ischemic injury has waned, is there a future for approaches targeting the inflammatory response in myocardial infarction? Considering the crucial role of inflammatory mediators in cardiac repair and their direct involvement in adverse remodeling of the infarcted heart, the answer is a resounding yes. What we need, however, is a paradigm shift and a realistic approach.

Targeting specific inflammatory mediators may not salvage a significant number of cardiomyocytes in reperfused infarcts, but may protect from chamber dilation and adverse remodeling, the hallmarks of post-infarction heart failure. In mouse models, loss of IL-1 or MCP-1 signaling significantly attenuated chamber dilation without reducing the size of the infarct [21], [6]. Inflammation-induced adverse remodeling of the infarcted heart may be due to activation of matrix metalloproteinases and subsequent matrix loss, resulting in reduced tensile strength of the infarct, cardiomyocyte slippage and loss of essential matrix-derived homeostatic signals that may support cardiomyocyte survival and function. Thus, IL-1 or MCP-1 inhibition may be promising strategies to attenuate post-infarction remodeling. The encouraging early results of IL-1 inhibition with IL-1 receptor antagonist (anakinra) [63] in patients with myocardial infarction may suggest a promising role of these approaches in prevention of adverse remodeling.

Translation of these concepts in the clinic requires recognition of the pathophysiologic complexity of post-infarction remodeling. After an acute infarction, patients have very different responses that may be, to some extent, independent of the size of the infarct. Some patients exhibit marked chamber dilation and rapid progression to systolic dysfunction and heart failure, whereas others develop predominant fibrosis associated with diastolic dysfunction. Age, gender, genetic predisposition and comorbid conditions (such as diabetes or hypertension) may influence the remodeling response. The subpopulation of patients exhibiting more extensive dilative remodeling may have prolonged or exaggerated myocardial inflammatory responses, possibly due to defects in negative regulation of the inflammatory reaction. These individuals may be excellent candidates for late implementation of targeted anti-inflammatory strategies in order to protect from the development of adverse remodeling. On the other hand, patients with predominant fibrosis and diastolic dysfunction (such as diabetics) [64] may exhibit overactive TGF- β signaling. These patients may benefit from therapies targeting the pro-fibrotic TGF- β /Smad signaling pathway [44]. Implementation of strategies targeting inflammatory mediators in patients with myocardial infarction will require identification of the predominant pathophysiologic process in each patient using a biomarker-based approach. For example, patients with prolonged increases in circulating levels of pro-inflammatory mediators (such as MCP-1) [65], [66] may have worse prognosis due to the development of dilative remodeling and may benefit from targeted inhibition of inflammation. On the other hand, biomarkers reflecting increased matrix synthesis [67] may identify individuals with predominant fibrosis. Thus, personalized, biomarker-based approaches may be needed to effectively target cardiac remodeling following myocardial infarction [68].

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Figure 1.

Initiation of the inflammatory response following myocardial infarction. Necrotic cardiomyocytes (CM) and damaged extracellular matrix release danger signals (DAMPs) triggering the complement cascade and activating TLR/IL-1 signaling. Resident mast cells (MC) degranulate and release preformed pro-inflammatory mediators, whereas activation endothelial cells (EC) and fibroblasts (Fib) results in induction of chemokines and cytokines. Leukocytes are recruited in the infarcted heart through activation of a multi-step adhesion cascade. Circulating neutrophils are captured by the activated endothelium (1) and roll on the endothelial surface (2) through interactions that involve the selectins. Neutrophils "sense" chemokines immobilized on the endothelial surface and exhibit integrins activation. Firm adhesion of the leukocyte to the endothelium follows through interactions that involve integrins and ICAM-1 (3). Transmigration of the neutrophils across the endothelial layer (4) is a complex process that involved the JAMs, ICAM-1 and VE-cadherin (see text). After extravasation into the infarct (5), neutrophils play an important role in clearance of the tissue from dead cells and matrix debris. (Additional symbol: ROS, reactive oxygen species).

Figure 2.

Resolution of the post-infarction inflammatory response involves activation of multiple inhibitory signals and recruitment, or transdifferentiation, of leukocyte subpopulations with suppressive properties. Expression of GDF-15 may reduce neutrophil integrin (Int) activation, inhibiting adhesive interactions between leukocytes and the endothelium. Recruitment of regulatory T cells (Tregs) and inhibitory monocyte subpopulations through distinct chemokine (CC)/chemokine receptor interactions may result in infiltration of the infarct with anti-inflammatory mononuclear cells. Clearance of apoptotic neutrophils by macrophages may result in secretion of inhibitory mediators, including IL-10, TGF- β and lipoxins. Infarct macrophages exhibit activation of endogenous signals (such as IRAK-M) that suppress inflammatory and immune responses (see text).

Table 1

Experimental studies on the effects of anti-integrin approaches in myocardial infarction

Experimental study	Type and timing of intervention	Experimental ischemia/reperfusion protocol	Outcome	Animal model
[52]	Anti-CD18 antibody (10 min before reperfusion)	90 min occlusion/48 h reperfusion	Nearly 50% reduction in infarct size	Dog
[53]	Anti-CD18 antibody (20 min before reperfusion)	90 min occlusion/4h reperfusion	40% reduction in infarct size	Baboon
[54]	Anti-CD18 antibody (first 5 min of reperfusion)	20 min occlusion/45 min reperfusion	92.9% recovery of coronary flow and 71% recovery of left ventricular pressure	Rat
[55]	Anti-CD18 antibody (10 min before reperfusion)	90 min occlusion/4.5h reperfusion	90% reduction in the necrotic area	Cat
[30]	Anti-CD11b/CD18 antibody (10 min after occlusion)	90min occlusion/6h reperfusion	46% reduction in infarct size	Dog
[56]	Anti-CD11b/CD18 antibody (45min after occlusion and at 12, 24, 36, 48h after reperfusion)	90min occlusion/72h reperfusion	37% reduction in infarct size	Dog
[69]	Anti-CD18 antibody (First dose: for 30min ending 30min before the onset of ischemia, second dose: for 30min starting 30min after reperfusion)	90min occlusion/3h reperfusion	No effect on infarct size	Dog

Table 2
Clinical trials testing the effects of anti-integrin approaches in patients with myocardial infarction

Trial	Type of intervention	Clinical Setting [number of patients]	Route and timing of administration	Endpoints	Outcome	Reference
LIMIT-AMI	Anti-CD18 antibody	ST elevation myocardial infarction patients within 12h from the onset of chest pain [n=394]	Intravenously before or soon after administration of tissue plasminogen activator [thrombolysis].	Primary: corrected TIMI frame count at 90min post angiography Secondary: percent TIMI grade flow at 90min, infarct size, ST elevation resolution	No effect on infarct size, TIMI flow, ST elevation resolution.	[57]
FESTIVAL	Anti-CD11/CD18 antibody	ST elevation myocardial infarction patients within 6h from the onset of chest pain. [n=60]	Intravenously after angioplasty	Clinical safety and tolerance of the antibody. Infarct size was measured by SPECT	No increase in side effects, no effect on infarct size	[59]
HALT-MI	Anti-CD11/CD18 antibody	ST elevation myocardial infarction patients within 6h from the onset of chest pain. [n=420]	Intravenously before angioplasty	Infarct size [measured by SPECT] 5–9 days later.	No effect on infarct size	[58]