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Cardiovascular Research 53 (2002) 31–47

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

The inflammatory response in myocardial infarction

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Received 20 April 2001; accepted 18 July 2001

Abstract

One of the major therapeutic goals of modern cardiology is to design strategies aimed at minimizing myocardial necrosis and optimizing cardiac repair following myocardial infarction. However, a sound understanding of the biology is necessary before a specific intervention is pursued on a therapeutic basis. This review summarizes our current understanding of the cellular and molecular mechanisms regulating the inflammatory response following myocardial ischemia and reperfusion. Myocardial necrosis induces complement activation and free radical generation, triggering a cytokine cascade initiated by Tumor Necrosis Factor (TNF)-α release. If reperfusion of the infarcted area is initiated, it is attended by an intense inflammatory reaction. Interleukin (IL)-8 synthesis and C5a activation have a crucial role in recruiting neutrophils in the ischemic and reperfused myocardium. Neutrophil infiltration is regulated through a complex sequence of molecular steps involving the selectins and the integrins, which mediate leukocyte rolling and adhesion to the endothelium. Marginated neutrophils exert potent cytotoxic effects through the release of proteolytic enzymes and the adhesion with Intercellular Adhesion Molecule (ICAM)-1 expressing cardiomyocytes. Despite this potential injury, substantial evidence suggests that reperfusion enhances cardiac repair improving patient survival; this effect may be in part related to the inflammatory response. Monocyte Chemoattractant Protein (MCP)-1 is also markedly upregulated in the infarcted myocardium inducing recruitment of mononuclear cells in the injured areas. Monocyte-derived macrophages and mast cells may produce cytokines and growth factors necessary for fibroblast proliferation and neovascularization, leading to effective repair and scar formation. At this stage expression of inhibitory cytokines such as IL-10 may have a role in suppressing the acute inflammatory response and in regulating extracellular matrix metabolism. Fibroblasts in the healing scar undergo phenotypic changes expressing smooth muscle cell markers. Our previous review in this journal focused almost exclusively on reduction of the inflammatory injury. The current update is prompted by the potential therapeutic opportunity that the open vessel offers. By promoting more effective tissue repair, it may be possible to reduce the deleterious remodeling, that is the leading cause of heart failure and death. Elucidating the complex interactions and regulatory mechanisms responsible for cardiac repair may allow us to design effective inflammation-related interventions for the treatment of myocardial infarction.

Keywords: Cytokines; Infarction; Infection/inflammation; Ischemia; Reperfusion

Abbreviations: α-SMAc, α-Smooth Muscle Actin; bFGF, basic Fibroblast Growth Factor; CSIF, Cytokine Synthesis Inhibitory Factor; DCFH, dichlorofluorescein; ICAM-1, Intercellular Adhesion Molecule-1; IL, interleukin; IP-10, Interferon γ-Inducible Protein-10; LFA-1, Leukocyte Function Antigen-1; LPS, lipopolysaccharide; LTB4, Leukotriene B4; LTC4, Leukotriene C4; MCP-1, Monocyte Chemoattractant Protein-1; M-CSF, Macrophage Colony-Stimulating Factor; MMP, matrix metalloproteinase; NADP, nicotineamide-adenine dinucleotide phosphate; NF-κB, Nuclear Factor-κB; PAF, Platelet Activating Factor; PAF-AH, Platelet Activating Factor-Acetylhydrolase; PDGF, Platelet-Derived Growth Factor; PSGL-1, P-Selectin Glycoprotein Ligand-1; ROS, reactive oxygen species; SCF, Stem Cell Factor; sCR1, Soluble Complement Receptor Type 1; SMemb, embryonic isoform of smooth muscle myosin heavy chain; SOD, superoxide dismutase; TIMP-1, Tissue Inhibitor of Metalloproteinases-1; TGF-β, Transforming Growth Factor-β; TNF-α, Tumor Necrosis Factor-α; TNFR, Tumor Necrosis Factor Receptor; u-PA, Urokinase-type Plasminogen Activator; VEGF, Vascular Endothelial Growth Factor; VLA-5, Very Late Antigen-5

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Time for primary review 19 days.

0008-6363/02/$ – see front matter
PII: S0008-6363(01)00434-5

1. Introduction

Myocardial infarction is associated with an inflammatory reaction, which is a prerequisite for healing and scar formation [1–4]. Coronary artery occlusion critically reduces blood flow to the portion of the myocardium subserved, markedly impairing the energy metabolism. In occlusions of the coronary arteries as short as 5 min, functional abnormalities of the reperfused myocardium are observed for as long as 24–48 h. These abnormalities are not attended by lethal injury and the ischemic myocardium ultimately recovers. This transient functional abnormality ('stunned myocardium') is related to reactive oxygen formation [5,6] but shows little if any evidence of an inflammatory reaction. However, ischemia of significant duration to induce infarction does result in an inflammatory response; this response is both accelerated and augmented if the ischemic tissue is reperfused.

The first experimental evidence that inflammation can extend myocardial injury came as the result of implementing anti-inflammatory strategies in animal models of myocardial ischemia and reperfusion. The systemic administration of corticosteroids was shown to decrease infarct size in a canine model of experimental myocardial infarction [7]. This early evidence led to a clinical study using methylprednisolone in patients with acute myocardial infarction, which resulted in catastrophic results, increasing the incidence of ventricular arrhythmias and extending infarct size [8]. Subsequent investigations suggested that corticosteroids inhibit the inflammatory process decreasing the number of infiltrating leukocytes, but also delay healing and collagen deposition [9]. This observation has been augmented by substantial evidence that reperfusion improves tissue repair and that this effect is mediated by enhancement of the inflammatory response [10–14]. Thus, there is a need for a better understanding of the cellular and molecular events associated with myocardial ischemia and reperfusion in order to develop more site-specific interventions that could mitigate inflammatory injury during early reperfusion without interfering with myocardial healing.

Subsequent experimental studies used various approaches to inhibit the inflammatory response in myocardial infarcts: reducing the generation of chemotactic factors by complement depletion [15], or administration of lipoxygenase inhibitors [16] and leukotriene B_4 (LTB4) antagonists were successful in limiting infarct size. Approaches that reduced neutrophil number such as anti-neutrophil antibodies [17], neutrophil depleting antimetabolites [18] or neutrophil filters [19] were also successful in reducing ischemia-related injury in some models. Finally, free radical scavengers, expected to protect against neutrophil-derived reactive oxygen species were also effective in reducing infarct size or sensitivity to ischemia [20].

This review highlights the mechanisms responsible for the regulation of the inflammatory response following experimental myocardial infarction. This process is dependent on a complex interaction between a variety of pleiotropic inflammatory mediators. Understanding of the basic mechanisms regulating the reaction to injury is crucial for the development of site-specific cell biological strategies of intervention to both reduce injury and promote repair.

2. Humoral inflammatory response

2.1. Initiation of the inflammatory process

2.1.1. Complement activation

Hill and Ward [21] were the first to demonstrate that ischemic myocardial injury can activate the complement cascade in a rat model of myocardial infarction. Subsequently Pinckard and colleagues [22] suggested that myocardial cell necrosis results in the release of subcellular membrane constituents rich in mitochondria, which are capable of triggering the early acting components (C1, C4, C2 and C3) of the complement cascade. Rossen and colleagues [23] have suggested that during myocardial ischemia, mitochondria, extruded through breaks in the sarcolemma, unfold and release membrane fragments rich in cardiolipin and protein. By binding C1 and supplying sites for the assembly of later acting complement components, these subcellular fragments provide the means to disseminate the complement-mediated inflammatory response to ischemic injury. mRNA and proteins for all the components of the classical complement pathway are upregulated in areas of myocardial infarcts [24,25]. Complement activation may have an important role in mediating neutrophil and monocyte recruitment in the injured myocardium. Dreyer and coworkers [26] showed that postischemic cardiac lymph contains leukocyte chemotactic activity, which is maximal during the first hour of reperfusion with washout within the next 3 h. Neutralizing antibodies to C5a added in vitro completely inhibited the chemotactic activity of postischemic cardiac lymph during that period. Other studies demonstrated that monocyte chemotactic activity in cardiac lymph collected in the first hour of reperfusion is wholly attributable to C5a [27].

Blocking activation of the complement system can be achieved by consumptive depletion (such as with cobra venom factor injection), by antibody-induced inhibition of individual complement components (e.g. C5), or by infusion of the soluble form of complement receptor type 1 (sCR1) [28]. Complement depletion using cobra venom factor injection at the time of experimental coronary artery occlusion has been shown to attenuate myocardial necrosis in a variety of animal models [15,29]. However, conclusions derived from these studies with a focus on complement depletion overlook the prior systemic activation that may result in deactivation of neutrophils. Weisman and

coworkers have demonstrated that infusion of soluble human complement receptor type 1 (sCR1) significantly decreased infarct size in a rat model of myocardial ischemia and reperfusion [30]. This study raises the possibility that interference with precisely targeted products of the complement system may reduce myocardial injury [31,32].

2.1.2. Reactive oxygen species

Reactive oxygen species (ROS) are molecules with unpaired electrons in their outer orbit. They have the potential to directly injure cardiac myocytes and vascular cells and may be involved in triggering inflammatory cascades through the induction of cytokines [33,34]. Reactive oxygen species have been shown to exert a direct inhibitory effect on myocardial function in vivo and have a critical role in the pathogenesis of myocardial stunning [5]. In addition, Granger and colleagues [35] have provided evidence for a potential role of reactive oxygen in leukocyte chemotaxis. Potential mechanisms through which reactive oxygen intermediates may generate a leukotactic stimulus include complement activation [36], induction of P-selectin expression [37,38], chemokine upregulation [39,40], and increase in the ability of endothelial ICAM-1 to bind to neutrophils [41].

Most of the evidence implicating ROS in the pathophysiology of myocardial infarction is derived from investigations using free radical scavengers. Jolly and coworkers [20] demonstrated that the combination of the antioxidant enzymes superoxide dismutase and catalase significantly reduced infarct size in dogs undergoing experimental myocardial ischemia and reperfusion. Other investigators found similar beneficial effects of antioxidant interventions in experimental models of myocardial infarction. However, there is a significant number of studies describing a failure of antioxidants to prevent injury or demonstrating an early protective effect, that waned with increased duration of reperfusion [42–44]. Recently, transgenic mice that overexpress copper, zinc superoxide dismutase (SOD1) showed significant protection from postischemic injury [45]. In addition, mice overexpressing MnSOD demonstrated a significant decrease in infarct size in Langendorf-perfused hearts undergoing left coronary artery ligation [46]. Unfortunately, two clinical studies using recombinant human superoxide dismutase in patients with acute myocardial infarction undergoing thrombolysis [47] or balloon angioplasty [48] demonstrated no significant improvement in left ventricular function. Both studies had a small sample size. In addition, prolonged coronary occlusion ($>$2 h) is usually present in the clinical setting of reperfused myocardial infarction and may cause extensive irreversible myocardial damage, leaving fewer myocytes to be affected by free radical-mediated injury [33,49].

2.1.3. The cytokine cascade

Experimental myocardial infarction is associated with the coordinated activation of a series of cytokine and adhesion molecule genes. A critical element in the regulation of these genes involves the complex formed by NF-κB and Iκβ [50]. NF-κB is activated by a vast number of agents, including cytokines (such as TNF-α and IL-1β) and free radicals. The genes regulated by the NF-κB family of transcription factors are diverse and include those involved in the inflammatory response, cell adhesion and growth control [51]. NF-κB activation has been demonstrated in various models of experimental myocardial ischemia and reperfusion [52–54]. Recently, in vivo transfer of NF-κB decoy oligodeoxynucleotides to bind the transcriptional factor, blocking inflammatory gene activation, reduced the extent of myocardial infarction following reperfusion [55].

The mechanisms responsible for triggering the cytokine cascade in the infarcted myocardium have only recently been investigated. Studies from our laboratory [56–58] indicated a role for preformed mast cell-derived mediators in initiating the cytokine cascade ultimately responsible for ICAM-1 induction in the reperfused canine myocardium. Mast cells have been recognized as an important source of preformed and newly synthesized cytokines, chemokines and growth factors. Gordon and Galli [59,60] identified mouse peritoneal mast cells as an important source of both preformed and immunologically-induced TNF-α. The constitutive presence of TNF-α in canine cardiac mast cells led us to postulate that mast cell-derived TNF-α may be released following myocardial ischemia, representing an ‘upstream’ cytokine responsible for initiating the inflammatory cascade.

We used a canine model of circumflex coronary occlusion and reperfusion, developed in our laboratory [61] that allows collection of cardiac lymph from chronically instrumented animals, in which inflammatory sequelae of the instrumentation surgery have dissipated. Our experiments demonstrated a rapid release of histamine and TNF-α bioactivity in the early post-ischemic cardiac lymph [56]. In addition, histochemical studies indicated mast cell degranulation in ischemic, but not in control sections of canine myocardium. These findings suggested rapid mast cell degranulation and mediator release following myocardial ischemia. C5a, adenosine and reactive oxygen may represent the stimuli responsible for initiation of mast cell degranulation. Furthermore, in vitro experiments showed that early post-ischemic cardiac lymph is capable of inducing IL-6 expression in canine mononuclear cells. Incubation with a neutralizing antibody to TNF-α in part inhibited IL-6 upregulation suggesting an important role for TNF-α as the upstream cytokine inducer. Mast cell degranulation appears to be confined in the ischemic area and results in rapid release of TNF-α, inducing IL-6 in infiltrating mononuclear cells.

Obviously, the role of TNF-α in myocardial infarction is much more complex than simply serving as a trigger of a cytokine cascade [62,63]. Recent experiments investigated

the role of TNF-α signaling in the infarcted myocardium using mice lacking TNF receptors [64]. TNFR1/TNFR2 double receptor knockout mice undergoing left coronary artery ligation had significantly higher infarct size and increased myocyte apoptosis when compared with wild-type controls [64]. These findings suggested that TNF-α may induce a cytoprotective signal capable of preventing or delaying the development of myocyte apoptosis following myocardial infarction.

Other studies have documented the persistent expression of TNF-α in a model of left anterior descending artery coronary occlusion in the rat [65]. TNF-α expression during the healing phase was not confined to the infarct or peri-infarct zone, but was also localized in the normal non-infarcted myocardium, in which remodeling was ongoing. Sustained TNF-α expression may have a role in the reparative process following myocardial infarction [66].

3. Cell-mediated inflammatory response

3.1. Neutrophil infiltration in reperfused myocardial infarcts

Neutrophil depletion in animals undergoing reperfused myocardial infarction led to a marked decrease in infarct size [17] suggesting that a significant amount of myocardial injury induced by coronary artery occlusion followed by reperfusion may be neutrophil-dependent [67,68]. Neutrophils may release oxidants and proteases and possibly express mediators capable of amplifying cell recruitment [69–71].

One of the earliest sequelae of reperfusion involves neutrophil trapping in the microvasculature. Engler and coworkers [19,72] demonstrated that entrapment of leukocytes in the microcirculation precedes their role in an inflammatory reaction. Neutrophils are large and stiff cells and may adhere to capillary endothelium preventing reperfusion of capillaries following coronary ischemia. The mechanism by which neutrophil trapping occurs in the microvessels is likely to be multifactorial. Chemotactic factors rapidly induce neutrophils to change shape and to become less deformable. Neutrophils also release a variety of autacoids, which induce vasoconstriction and platelet aggregation, such as thromboxane B_2 and LTB_4. Neutrophil interaction with endothelial cells via specific adhesion molecules results in their margination and adhesion to the endothelium. The most dramatic and pathologically significant microvascular abnormality is known as the no reflow phenomenon and has also been directly linked to neutrophil localization. Ambrosio and coworkers [73,74] demonstrated in a canine model that the occurrence of areas of markedly impaired perfusion in postischemic myocardium is related only in part to an inability to reperfuse certain areas on reflow. The delayed, progressive fall in flow to areas that initially received adequate reperfusion appeared to be a more important factor. This phenomenon develops in regions receiving no collateral flow during ischemia and is associated with neutrophil accumulation and capillary plugging during late reperfusion.

While changes in cell shape and deformability, and vasoconstriction are important mechanisms for neutrophil accumulation in the ischemic and reperfused myocardium, the bulk of evidence suggests that the more specific interactions between adhesion molecules are the most critical factors in control of neutrophil-induced pathophysiological changes.

3.2. Neutrophil–endothelial interactions and neutrophil transmigration following myocardial ischemia and reperfusion

A better understanding of the molecular interactions between leukocytes and endothelium has given rise to a consensus model of how leukocyte recruitment into tissues is regulated [75,76]. There is increasing evidence that leukocyte–endothelial interactions are regulated by a cascade of molecular steps that correspond to the morphological changes that accompany adhesion. This adhesion cascade has been divided into sequential steps based on visual assessment of the post-capillary venules during the early stages of acute inflammation. In the absence of inflammation, leukocytes are rarely seen to interact with the vessel wall. After the inflammatory stimulus is applied, leukocytes roll along the post-capillary venules (but not arterioles or small arteries) at velocities distinctly below that of flowing blood. Some rolling cells can be seen to arrest and after a few minutes change shape in apparent response to local chemotactic stimuli. Extravasation into the extravascular tissue follows (Fig. 1). Each of these steps requires either upregulation or activation of distinct sets of adhesion molecules (Fig. 2).

3.2.1. Neutrophil rolling: the role of the selectins

The selectin family of adhesion molecules mediates the initial capture of leukocytes from the rapidly flowing bloodstream to the blood vessel, before their firm adhesion and diapedesis at sites of tissue injury and inflammation [77–79]. The selectin family consists of three closely related cell-surface molecules: L-selectin (CD62L), E-selectin (CD62E), and P-selectin (GMP-140, CD62P). The individual members of the selectin group were designated by prefixes, which were chosen according to the cell type where the molecule was first identified. L-selectin expression is limited to hematopoietic cells, with most classes of leukocytes constitutively expressing L-selectin at some stage of differentiation. The majority of circulating neutrophils, monocytes, eosinophils, T cells and B cells express L-selectin, which is rapidly shed from the surface of these cells following their activation. The broad expression of L-selectin allows it to play a role in the trafficking of all

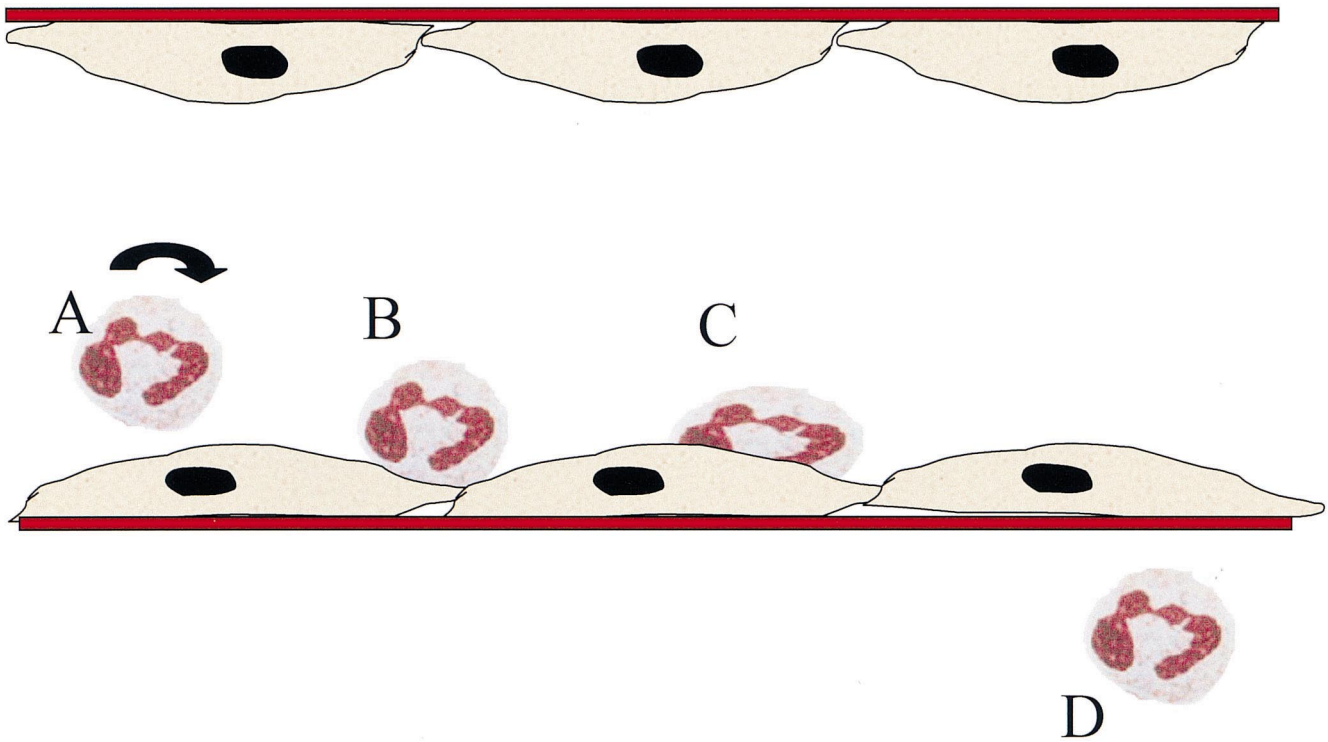

Fig. 1. Diagram illustrating the sequential steps of the adhesion cascade regulating neutrophil localization in post-capillary venules during the early stages of acute inflammation. (A) After the inflammatory stimulus is applied, leukocytes roll along the post-capillary venules at velocities distinctly below that of flowing blood. (B) Some rolling cells can be seen to arrest and after a few minutes change shape (C) in apparent response to local chemotactic stimuli. Extravasation into the extravascular tissue follows (D). Each of these steps requires either upregulation or activation of distinct sets of adhesion molecules.

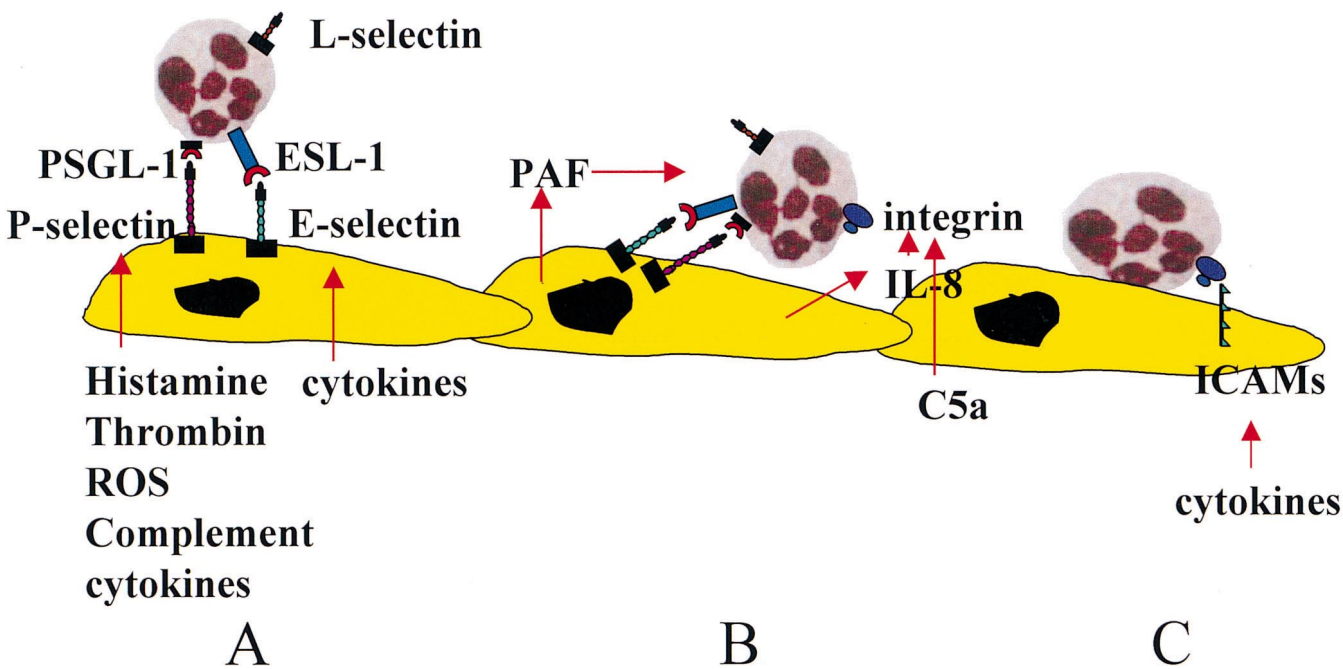

Fig. 2. Endothelial–neutrophil interactions leading to neutrophil transmigration into the injured myocardium. (A) The initial tethering of neutrophils to the endothelial cell surface is mediated by the selectins. (B) This enables the leukocyte to roll along the venular wall and to ‘sense’ activating factors (such as IL-8 and C5a). These interactions lead to neutrophil integrin activation. (C) Firm adhesion of the leukocyte is mediated through binding of neutrophil integrins to members of the immunoglobulin superfamily expressed in stimulated endothelial cells. Abbreviations: P-Selectin Glycoprotein Ligand-1, PSGL-1; E-Selectin Ligand-1, ESL-1; Reactive oxygen species, ROS; Platelet Activating Factor, PAF; Interleukin-8, IL-8; Intercellular Adhesion Molecule, ICAM.

leukocyte lineages. In contrast, E-selectin is expressed only following de novo synthesis 4–6 h after activation of endothelial cells by cytokines (such as TNF-α, IL-1β) or by bacterial endotoxin [80,81]. P-Selectin is constitutively found in Weibel–Palade bodies of endothelial cells and in α granules of platelets. Within minutes after activation by thrombogenic and inflammatory mediators, P-selectin is mobilized to the cell surface without the need for new protein synthesis. Inducing agents include thrombin, histamine, complement fragments, oxygen-derived free radicals, LTC4/D4 and cytokines. In addition to its regulation through transport to the cell surface, P-selectin is also inducible by cytokines and endotoxin both in vitro [82] and in vivo [83].

One important property of the selectins is that they promote leukocyte attachment and rolling at shear stresses characteristic of post-capillary venules. All three selectins are involved in leukocyte entry into tissues [84–86]. Recently, studies using transgenic mice have improved our understanding of the role of selectins in leukocyte trafficking. L-Selectin-deficient mice have shown a significant reduction in lymphocyte homing to peripheral lymph nodes and leukocyte infiltration to sites of inflammation [87,88]. P-Selectin-deficient mice demonstrated virtually total absence of rolling in mesenteric venules and delayed neutrophil recruitment to the peritoneal cavity upon experimentally-induced inflammation [89]. In contrast to P- and L-selectin mutants, E-selectin-deficient mice have no obvious abnormalities of the inflammatory response [90]. However, P-selectin blocking by treatment of the E-selectin-deficient animals with an anti-murine P-selectin antibody significantly inhibited neutrophil emigration in two distinct models of inflammation [90]. These findings suggest that P- and E-selectin may share overlapping functions [78].

The role of selectins in ischemia and reperfusion is not well defined at present and represents an area of active investigation. L-Selectin is constitutively expressed in neutrophils in a highly specific distribution and its shedding upon activation may be important for leukocyte recruitment [91,92]. P-Selectin surface expression occurs rapidly on endothelial cells under circumstances likely to be seen during ischemia and reperfusion. It is stored in the Weibel–Palade bodies and is rapidly translocated to the endothelial surface in response to thrombin and/or oxidative stress, both of which would be likely to be found upon reperfusion and initiated by thrombolytic agents, and to histamine, which is rapidly released in the ischemic and reperfused myocardium by degranulating mast cells. Experimental studies have suggested that monoclonal antibodies against L-selectin [93] and P-selectin [94] were effective in reducing myocardial necrosis, preserving coronary endothelial function and attenuating neutrophil accumulation in ischemic myocardial tissue in a feline model of ischemia/reperfusion.

Unlike most other cell adhesion molecules that bind to their ligands on the basis of protein–protein interactions, the ligands of the selectins are composed of a scaffold protein, or a lipid carrier molecule, which is modified by certain carbohydrates [95]. Numerous reports have documented that glycolipids bind specifically to the selectins. Sialyl Lewis^{x}-carrying glycolipids and sialyl Lewis^{a}-carrying neoglycolipids were shown to support rolling of E-selectin-transfected cells and of L-selectin-expressing leukocytes [96]. P-Selectin glycoprotein ligand-1 (PSGL-1) is the best-characterized selectin ligand to date, fulfilling all criteria for a physiologically relevant ligand [97]. It is the major ligand for P-selectin on human neutrophils as the monoclonal antibody PL-1 to PSGL-1 completely inhibits neutrophil rolling on P-selectin [98]. Recently, peptide analogs of PSGL-1 have been constructed. A soluble form of PSGL-1 has been found to be protective in experimental renal ischemia and reperfusion in rats decreasing renal necrosis due to neutrophil infiltration [99]. Furthermore, a recombinant analog of sPSGL-1 significantly reduced myocardial necrosis in a feline model of coronary occlusion and reperfusion [100].

Recent experiments suggested that P-selectin-deficient mice show decreased infarct size after 30 min of coronary occlusion and 2 h of reperfusion [101]. In contrast, no difference in infarct size was noted after a 60-min ischemic period [101]. In addition, mice with a combined P-selectin and ICAM-1 deficiency demonstrated impaired neutrophil trafficking without a difference in infarct size due to myocardial ischemia and reperfusion [102].

Thus, current concepts suggest a role for selectins in supporting margination under shear stress following experimental myocardial ischemia and reperfusion. The transient nature of this adhesive interaction is important since it allows leukocytes to ‘sample’ the local endothelium for the presence of specific trigger factors that can activate leukocyte integrins and allow the cascade to proceed.

3.2.2. CD18 and the leukocyte beta 2 integrins

Although rolling appears to be a prerequisite for eventual firm adherence to blood vessels under conditions of flow, selectin-dependent adhesion of leukocytes does not lead to firm adhesion and transmigration unless another set of adhesion molecules, the integrins, is engaged. Integrins are a family of heterodimeric membrane glycoproteins that consist of an α and a β subunit; these subunits are associated through noncovalent bonds and transported to the cell surface as a complex [103]. For neutrophils, firm adhesion requires activation of the β2 (CD18) integrins, which share the beta chain CD18 paired with CD11a (LFA-1), CD11b (Mac-1), or CD11c (p150,95). This results in binding to one of the intercellular adhesion molecules on the surfaces of endothelial cells. LFA-1, Mac-1 and p150,95 have different and yet overlapping roles in adhesion, in part due to their characteristics of expression on leukocytes.

Stationary neutrophils adherent to the luminal endothelial surface frequently change shape and assume the characteristic bipolar configuration of motile cells [104,105]. This event may result from interaction with surface-bound chemokines [106]. Transendothelial migration follows and leads to neutrophil infiltration in the inflamed tissues. Antibodies that inhibit LFA-1 adhesion are effective in blocking transmigration [107] and LFA-1-deficient mice show dramatically decreased neutrophil extravasation at sites of inflammation [108]. In contrast, antibodies that block Mac-1 adhesion are marginally effective [107] and Mac-1-deficient mice demonstrate no deficit in neutrophil emigration [109]. These findings suggest that LFA-1 and not Mac-1 is critical for neutrophil extravasation in sites of inflammation.

Integrin-related strategies have been used to mitigate the inflammatory process by numerous investigators. Simpson and colleagues showed in a canine model that a monoclonal antibody against CD11b substantially reduced myocardial necrosis after 90 min of coronary ischemia and 6 h of reperfusion [110]. Later experiments suggested that inhibition of Mac-1-mediated neutrophil adhesion may provide sustained limitation of myocardial necrosis by demonstrating substantial benefit in a canine model of 90 min of coronary ischemia and 72 h of reperfusion when an F(ab′)2 fragment of a monoclonal antibody against CD11b was tested [111]. In a canine model of experimental myocardial infarction, anti-CD18 antibodies have been shown to reduce infarct size and preserve left ventricular function [112]. Additional studies suggested that the effectiveness of different anti-CD18 antibodies in preventing injury is highly dependent on the specific antibody employed [113]. In addition, CD18-deficient mice demonstrated a significant reduction in neutrophil accumulation and myocardial necrosis following ischemia and reperfusion [114].

3.2.3. Neutrophil chemotaxis in myocardial infarction: the role of chemokines

Complement activation has an important role in neutrophil chemotaxis in the infarcted myocardium. Over the past decade, a superfamily of polypeptide leukocyte chemoattractants, known as chemokines, have been demonstrated to induce rapid and selective leukocyte transmigration [115–117]. Four chemokine subfamilies (CXC, CC, C and CX3C) are classified based on their primary aminoacid sequences. The CXC chemokine Interleukin-8 (IL-8) appears to have a fundamental role in regulating neutrophil localization in ischemic tissues. Sekido and coworkers [118] demonstrated that reperfusion of ischemic lung caused neutrophil infiltration and destruction of pulmonary structure, as well as local production of IL-8. Furthermore, the administration of a neutralizing monoclonal antibody against IL-8 prevented neutrophil infiltration and tissue injury, proving a crucial role of locally produced IL-8 in this model.

In reperfused myocardial infarcts, IL-8 is markedly and consistently induced after 1 h of reperfusion and persists at high levels beyond 24 h [119]. In addition, recombinant canine IL-8 markedly increased adhesion of neutrophils to isolated canine cardiac myocytes. This adhesion resulted in direct cytotoxicity for cardiac myocytes [119].

Other studies [120] using a rabbit model of myocardial infarction suggested a sequential release of chemoattractants: the first, C5a is generated in interstitial fluid, followed by IL-8 predominantly synthesized by infiltrating neutrophils.

Lipid-derived mediators may also be important factors in regulating neutrophil accumulation following myocardial infarction. In many species, activated neutrophils release LTB4, which functions as a potent chemotactic agent. Platelet Activating Factor (PAF) is a phospholipid mediator that belongs to a family of biologically active, structurally related alkyl phosphoglycerides [121]. PAF is formed by endothelial cells in response to thrombin and acts as a potent chemotactic agent promoting neutrophil adhesion to endothelial cells [122]. A recent study showed that inhibition of PAF through enzymatic hydrolysis with PAF acetylhydrolase (PAF-AH) significantly decreased neutrophil infiltration in a rabbit model of experimental myocardial infarction, improving systolic function [123].

4. Mechanisms of neutrophil-induced myocardial injury

The focus of the previous sections of this review has been the mechanism by which neutrophils are attracted to and activated in the ischemic and reperfused myocardium. The mechanism by which neutrophil-induced myocardial injury occurs has only recently been investigated. In addition to the potential role of neutrophil-mediated microvascular obstruction cited above, there is also substantial evidence suggesting that neutrophils may directly injure parenchymal cells through release of specific toxic products [124]. Obviously, neutrophils accumulating in the ischemic and reperfused areas might release proteolytic enzymes or reactive oxygen species to injure surrounding myocytes. However, under conditions found in vivo, these toxic products are almost exclusively secreted by adherent neutrophils. Thus, it appears that a ligand-specific adhesion of the neutrophils to the cardiac myocytes may be critical for the mediation of ischemia-induced myocyte injury.

4.1. Adhesion-dependent cytotoxicity

ICAM-1 is one of the primary ligands for the CD18 integrins [125]. However, in contrast to the restricted cellular distribution of the β2 integrins, ICAM-1 can be expressed by many cell types under certain circumstances. Studies from our laboratory examined the potential mechanisms of neutrophil adhesion to isolated adult canine

cardiac myocytes. Intercellular adhesion occurred only if the myocytes were stimulated with cytokines inducing ICAM-1 expression and when the neutrophils were stimulated to show Mac-1 activation. In vitro, myocyte ICAM-1 induction could be effected by the cytokines IL-1, TNF-α and IL-6; neutrophil activation could be effected by zymosan-activated serum (a source of C5a) PAF and IL-8. The binding of neutrophils to activated cardiac myocytes was found to be specific for Mac-1–ICAM-1 interaction, and was completely blocked by antibodies to ICAM-1, CD11b and CD18. This interaction was unaffected by antibodies to CD11a, which are capable of blocking neutrophil adhesion to an endothelial cell monolayer. Adhering neutrophils were apparently cytotoxic, as indicated by the sustained contraction often observed in myocytes after neutrophil adhesion.

In other experiments, the mechanisms of neutrophil-induced cytotoxicity were studied [126]. Either neutrophils or cardiac myocytes were loaded with 2′,7′-dichlorofluorescein (DCFH), and the adherence-dependent oxidation of this marker to DCF was monitored under fluorescence microscopy. Using zymosan-activated serum to activate the neutrophils in the presence of cytokine-stimulated cardiac myocytes, neutrophil–myocyte adhesion ensued as described above. When neutrophils were loaded with DCFH, fluorescence appeared almost immediately upon adhesion of the neutrophil to a myocyte suggesting a rapid adhesion-dependent activation of the NADP oxidase system of the neutrophil. In contrast, fluorescence of the cardiac myocytes appeared after several minutes and was rapidly followed by irreversible myocyte contracture. The iron chelator desferrioxamine and the hydroxyl radical scavenger, dimethylthiourea, did not inhibit neutrophil adherence, but completely inhibited the fluorescence and contracture seen in the cardiac myocyte, preventing the neutrophil-mediated injury. In contrast, extracellular oxygen radical scavengers such as superoxide dismutase and catalase or extracellular iron chelators such as starch-immobilized desferrioxamine did not inhibit fluorescence, adhesion or cytotoxicity. Under these experimental conditions, no superoxide production could be detected in the extracellular medium during the neutrophil–myocyte adhesion. These data suggest that Mac-1/ICAM-1 adherence activates the neutrophil respiratory burst resulting in a highly compartmented iron-dependent myocyte oxidative injury.

4.2. Inflammatory myocardial injury in vivo — Possible role of ICAM-1

The pertinence of the in vitro neutrophil-mediated myocyte injury to ischemia/reperfusion injury was suggested by experiments with postischemic cardiac lymph which demonstrate the appearance of C5a activity present during the first 4 h of reperfusion along with neutrophils showing upregulation of Mac-1 on their surface. Postischemic cardiac lymph also contained cytokine activity that upregulated ICAM-1 in isolated cardiac myocytes; this latter activity was neutralized by antibodies to human IL-6 [127]. Further studies were designed to directly evaluate the role of ICAM-1 in myocardial inflammation associated with ischemia and reperfusion.

Using a canine model of reperfused myocardial infarction, Kukielka and coworkers [128] demonstrated ICAM-1 mRNA expression in ischemic myocardial segments as early as 1 h after reperfusion, with marked elevations after longer time intervals. No detectable ICAM-1 mRNA was found in segments with normal blood flow while in the previously ischemic areas, ICAM-1 mRNA appeared as an inverse function of coronary blood flow. At later time points such as 24 h, however, mRNA was found in all myocardial samples (although tissue expression of protein remains confined to the viable border zone), suggesting that circulating cytokines are inducing ICAM-1 mRNA in normal as well as in ischemic areas. The actual expression of ICAM-1 protein was not seen until 3–6 h and was almost exclusively seen in the ischemic area at all time points, implying the possibility of a posttranscriptional regulation of ICAM-1 expression in cardiac myocytes, or, more likely, proteolytic solubilization of surface ICAM-1 on normal cells that may be defective in the jeopardized zone allowing the presence of surface ICAM-1.

Using in situ hybridization techniques, substantial message for ICAM-1 was detected in much of the reperfused viable myocardium by 1 h of reperfusion, adjacent to areas of contraction band necrosis [129]. At 3 h, ICAM-1 mRNA expression occurred in cells in the jeopardized area that appeared viable histologically. In contrast, under circumstances where reperfusion did not occur, ischemic segments did not express ICAM-1 mRNA or ICAM-1 protein in areas of occlusion for periods up to 24 h. It is important to point out that the layers of myocardial cells directly adjacent to the endocardium are spared injury, conserve glycogen and do not express ICAM-1 mRNA in early reperfusion, probably as a result of diffusion across the endocardium from the left ventricular chamber. In addition, this area of induction of ICAM-1 mRNA on the viable border zone region of the infarct is the area where the most intense neutrophil margination and infiltration occur.

Based on these observations, it is reasonable to propose that ICAM-1 facilitates both the emigration of neutrophils in reperfused myocardium and their adherence-dependent cytotoxic behavior. Constitutive levels of ICAM-1 on endothelial cells may be sufficient to support CD18-dependent adhesion and subsequent transendothelial migration in response to chemotactic stimuli, whereas newly expressed ICAM-1 may participate in the myocardial injury associated with reperfusion only under circumstances where a leukotactic gradient and neutrophil activation are present.

4.3. Mechanism of ICAM-1 induction

Because of the capacity of IL-6, present in postischemic

cardiac lymph, to induce myocyte ICAM-1 expression, the expression of IL-6 mRNA in the ischemic and reperfused myocardium was investigated. In these experiments it was demonstrated that IL-6 was rapidly expressed in the same ischemic segments in which ICAM-1 mRNA was found with a peak preceding that of ICAM-1 mRNA [130]. As with ICAM-1 the expression of IL-6 mRNA appeared to be dependent upon reperfusion.

These observations are consistent with the hypothesis that reperfusion initiates a cascade of cytokine-related events leading to IL-6 expression and subsequent induction of ICAM-1 mRNA in the ischemic and reperfused myocardium. It appears that IL-6 synthesis is rapidly induced in cells found within the ischemic and reperfused areas. Mononuclear cells and myocytes in the border zone of myocardial infarcts exhibit reperfusion-dependent expression of IL-6 mRNA within 1 h after reperfusion [131]. TNF-α of mast cell origin may be a crucial factor in upregulating IL-6 in infiltrating cells and initiating the cytokine cascade responsible for myocyte ICAM-1 induction and subsequent neutrophil-induced injury [56].

In addition, IL-6 effects may extend beyond the induction of ligand-specific adhesion of neutrophils to cardiac myocytes. Finkel and coworkers [132,133], have demonstrated that IL-6 may act as a nitric oxide-dependent cardiac depressant and may be associated with stunned myocardium. Furthermore, recent experiments indicated that IL-6 knockout mice demonstrate significantly delayed cutaneous wound healing suggesting a significant role for IL-6 in tissue repair [134].

5. The role of the inflammatory response in the healing myocardial infarction

The potential dangers of anti-inflammatory strategies described above have prompted extensive studies on the role of inflammation in cardiac repair. Both experimental and clinical evidence demonstrate that an open infarct vessel promotes repair even when reperfusion occurs when no myocardial tissue can be salvaged [11,12,135]. The role of reperfusion-induced inflammation in the repair process has been suggested in several experimental models [10,136]. Infiltrating mononuclear cells and mast cells appear to orchestrate the cardiac repair process through a complex cascade involving cytokines and growth factors (Fig. 3). The remainder of this review will focus on this aspect of the inflammatory response.

5.1. Mononuclear cell infiltration

Mononuclear cells infiltrate the infarcted myocardium in the first few hours of reperfusion. The mechanisms responsible for monocyte recruitment have recently been elucidated. Monocyte chemotactic activity in the first hour after reperfusion was wholly attributable to C5a [27]. Transforming growth factor (TGF)-β1 contributed significantly to this chemotactic activity after 60–180 min, and after 180 min, monocyte chemotactic activity in lymph was largely dependent on monocyte chemoattractant protein (MCP)-1 acting in concert with TGF-β1. MCP-1 was rapidly upregulated in the venular endothelium of ischemic myocardial segments. In the absence of reperfusion, no significant MCP-1 induction was noted [137]. Increased monocyte recruitment may lead to more effective healing, explaining the beneficial effects of late reperfusion, when no myocardial tissue can be salvaged.

A recent investigation examined the monocyte–tissue matrix interactions responsible for monocyte accumulation in the infarcted areas demonstrating that reperfusion of ischemic myocardium released diverse fibronectin fragments into cardiac extracellular fluids [138]. Cell-binding fibronectin fragments released under these circumstances induced the proteolysis of monocyte cell-surface Very Late Antigen (VLA)-5. This process appeared to be mediated by serine proteases activated in the course of the response to myocardial injury.

After recruitment in the infarcted territory monocytes differentiate into macrophages. Local upregulation of Macrophage Colony-Stimulating Factor (M-CSF) may have an important role in this process providing the milieu necessary for monocyte maturation [2]. The exact role of the macrophages in the healing scar has not been fully investigated, however they may serve as an important source of cytokines and growth factors [139]. In addition, they may regulate extracellular matrix metabolism through the synthesis of matrix metalloproteinases and their inhibitors [14,140].

5.2. IL-10 as a modulator of the inflammatory response

The inflammatory response ultimately leads to healing and repair of the injured territory. Thus, the molecular signals induced following myocardial infarction may mediate suppression of tissue injury and regulate scar formation. Interleukin-10 (IL-10), a cytokine initially described as cytokine synthesis inhibitory factor (CSIF) is primarily a product of activated Th2 cells and endotoxin-stimulated monocytes [141]. Among the different cell types affected by IL-10, monocyte-macrophages appear to be particularly modified in regard to their function, morphology and phenotype. IL-10 inhibits the production of IL-1α, IL-1β, TNF-α, IL-6 and IL-8 by LPS-activated monocytes, suppressing the inflammatory response. IL-10 also suppresses expression of IL-12, a cytokine primarily produced by activated monocytes and a dominant factor in directing Th1 type responses [141]. Furthermore IL-10 may have a significant role in extracellular matrix formation by modulating expression of metalloproteinases and their inhibitors [142]. The potential role of IL-10 in experimental myocardial infarction has recently been investigated [14,143]. IL-10 mRNA and protein upregulation was demonstrated in the reperfused infarcted myocardium using a canine

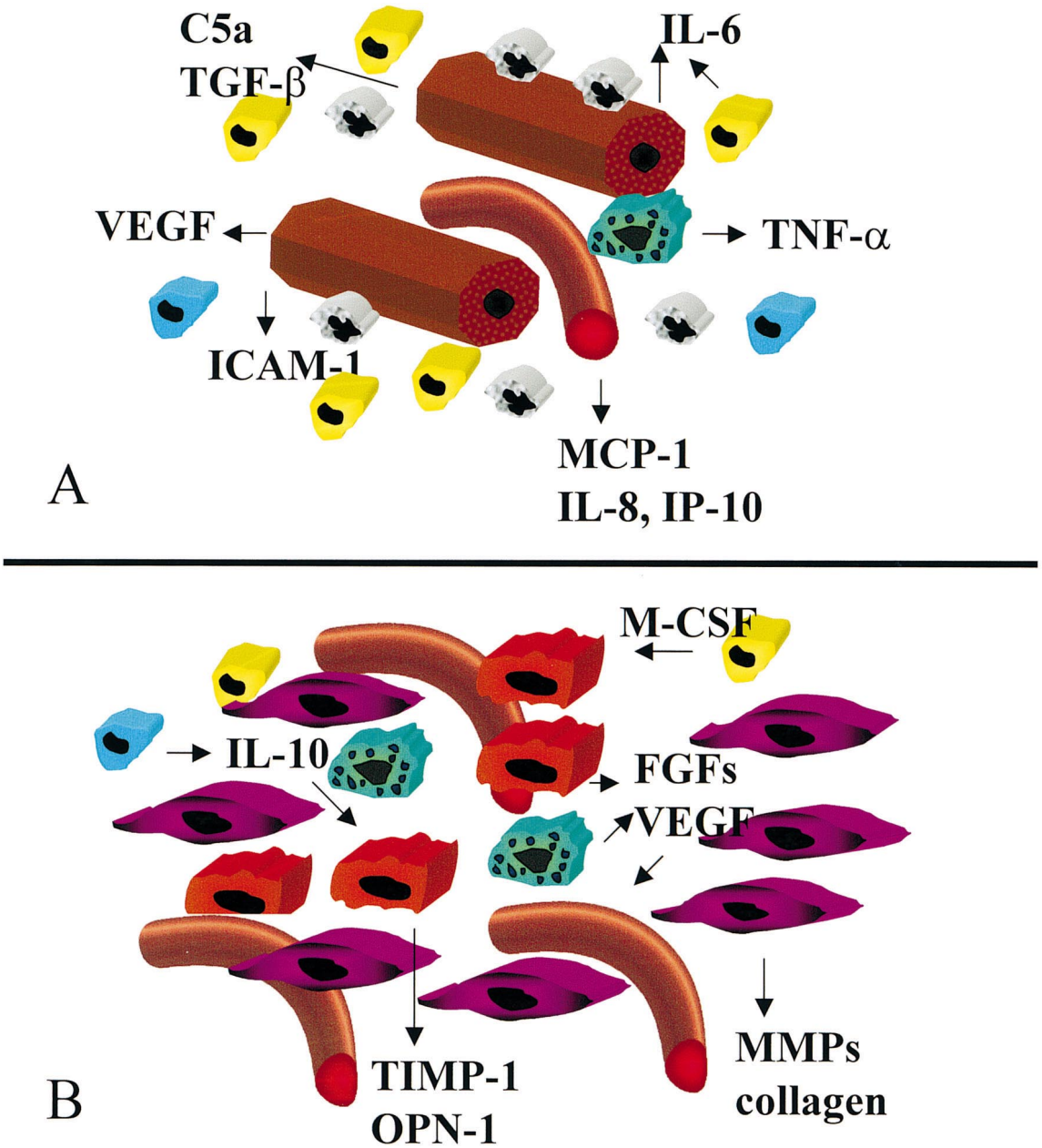

Fig. 3. Schematic diagram illustrating the cellular events associated with the inflammatory response in reperfused myocardial infarcts. (A) In the first 24 h of reperfusion the injured myocardium is infiltrated by neutrophils (gray), monocytes (yellow) and lymphocytes (cyan). Leukocyte recruitment is regulated by complement activation, release of bioactive TGF-β, and induction of chemokines (such as MCP-1 and IL-8). Resident mast cells (green) release preformed mediators (such as histamine and TNF-a) initiating the cytokine cascade, which leads to IL-6 synthesis in mononuclear cells and myocytes (brown). Subsequently, cytokine-stimulated myocytes in the ischemic border zone express ICAM-1 and may be susceptible to neutrophil-mediated cytotoxic injury. At this stage, both angiogenic (such as VEGF, IL-8, MCP-1) and angiostatic factors (such as IP-10) are released; thus the angiogenic process may be delayed until the wound is debrided and a fibrin-based provisional matrix is formed. (B) During the healing phase, infiltrating monocytes differentiate into macrophages (orange). The maturation process may be regulated by local synthesis of M-CSF. Macrophages and mast cells accumulate in the healing scar and secrete a variety of growth factors and cytokines, inducing fibroblast proliferation. Lymphocytes and a subset of the macrophages produce the macrophage-modulating cytokine IL-10, which may have a role in suppressing the inflammatory response and in tissue remodeling by regulating expression of metalloproteinases and their inhibitors. Fibroblasts undergo phenotypic changes expressing α-smooth muscle actin and produce collagen and other extracellular matrix components. At this stage, suppression of the angiostatic chemokine IP-10 may lead to active angiogenesis regulated by a variety of factors, such as VEGF, basic FGF and the angiopoetins. Abbreviations: Transforming Growth Factor-β, TGF-β; IL, Interleukin; Intercellular Adhesion Molecule-1, ICAM-1; Monocyte Chemoattractant Protein-1, MCP-1; Interferon-γ Inducible Protein-10, IP-10; Tumor Necrosis Factor-α, TNF-α; Vascular Endothelial Growth Factor, VEGF; Macrophage Colony-Stimulating Factor, M-CSF; Matrix Metalloproteinases, MMPs; Tissue Inhibitor of Metalloproteinases-1, TIMP-1; Osteopontin-1, OPN-1; Fibroblast Growth Factors, FGFs.

model of myocardial infarction. IL-10 expression was first detected at 5 h and peaked following 96–120 h of reperfusion. In contrast, IL-4 and IL-13, also associated with suppression of acute inflammation and macrophage deactivation, were not expressed. In the ischemic canine heart, CD5 positive lymphocytes were the predominant source of IL-10 in the myocardial infarct. In the absence of reperfusion, no significant induction of IL-10 mRNA was noted. In addition, IL-12, a Th1 related cytokine associated with macrophage activation, was not detected in the ischemic myocardium [14]. In vitro experiments demonstrated that late postischemic cardiac lymph induced Tissue Inhibitor of Metalloproteinases (TIMP)-1 mRNA expression in isolated canine mononuclear cells. This effect was inhibited when the incubation contained a neutralizing antibody to IL-10. These findings suggest that lymphocytes infiltrating the ischemic and reperfused myocardium express IL-10 and may have a significant role in healing by modulating mononuclear cell phenotype and inducing TIMP-1 expression. Furthermore, IL-10 was found to have a role in regulation of the angiogenic in human lymphoid malignancies [144] and in a model of ischemia-induced angiogenesis in mice hindlimb [145]. These studies underscore the importance of IL-10 in the healing process.

Additional investigations indicated that IL-10-deficient mice show an enhanced inflammatory response following experimental myocardial infarction, demonstrated by increased neutrophil recruitment, elevated plasma levels of TNF-α and high tissue expression of ICAM-1 [143]. Thus, IL-10 may have a protective role after myocardial ischemia/reperfusion through the suppression of the acute inflammatory process.

5.3. Mast cell accumulation in the healing scar

As described above, mast cell degranulation is an early source of preformed histamine and TNF-α, modulating the inflammatory response. In later stages, there is a likely role for mast cells in the superbly orchestrated interaction of cells, cytokines, growth factors and extracellular matrix proteins mediating myocardial repair. Macrophages and mast cells provide a rich source of cytokines and growth factors necessary to support fibroblast proliferation and neovessel formation. There is significant evidence that mast cells participate in the fibrotic process [146,147]. Recent studies demonstrated that mast cell numbers increase in the healing phase of reperfused canine myocardial infarcts [148]. The increase in mast cell density was first noted after 72 h of reperfusion. Following 5–7 days of reperfusion, mast cell numbers in fibrotic areas, in which myocytes were fully replaced by scar were markedly higher than the numbers from areas of the same section showing intact myocardium. These experiments failed to demonstrate significant numbers of proliferating mast cells in the healing heart. Although the contribution of mast cell proliferation cannot be ruled out, chemotaxis of circulating mast cell precursors in the healing myocardium may be the predominant mechanism responsible for mast cell accumulation in the ischemic myocardium. Mast cells originate from CD34+ hematopoietic stem cells and circulate as immature precursors in the peripheral blood. Rodewald and colleagues [149] identified a cell population in murine fetal blood that fulfills the criteria of progenitor mastocytes. It is defined by the phenotype Thy-1 (lo) c-kit (hi), expresses RNAs encoding mast cell associated proteases, but lacks expression of the high-affinity immunoglobulin E receptor.

The factors responsible for mast cell accumulation in areas of fibrosis remain to be defined. Stem Cell Factor (SCF) is a potent mast cell chemoattractant that stimulates directional motility of both mucosal- and connective tissue type-mast cells [150]. In addition, several angiogenic factors, such as Platelet-Derived Growth factor (PDGF), Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF) have been demonstrated to promote murine mast cell chemotaxis in vitro. However, SCF along with the anaphylatoxins C3a and C5a are the only factors shown to induce migration of human mast cells. Subcutaneous administration of recombinant human SCF to baboons produced a marked expansion of the mast cell population, which was reversed when the cytokine was discontinued [66], providing the first direct evidence that a specific factor can regulate mast cell development in vivo. In a canine model of myocardial infarction, SCF mRNA expression was markedly upregulated in ischemic myocardial segments following 1 h of ischemia and 72 h of reperfusion. At the same time point, an increase in mast cell numbers was noted in the healing myocardium. Immunohistochemical studies showed that SCF immunoreactivity in the healing myocardial scar was predominantly localized in a subset of macrophages. In addition to being a mast cell chemoattractant, SCF critically regulates the maturation and survival of mast cells by suppressing mast cell apoptosis, enhancing mast cell maturation and inducing mast cell adhesion to fibronectin. Furthermore, SCF is capable of inducing substantial mast cell histamine release and can promote the functional activation of mast cells in vivo. All these actions may be important in regulating mast cell growth and activity after myocardial ischemia. Recently, Patella and colleagues [151] demonstrated increased mast cell density and stem cell factor expression in patients with idiopathic and ischemic cardiomyopathy, suggesting that sustained mast cell hyperplasia in cardiomyopathic hearts may contribute to collagen accumulation and fibrosis.

The potential role of mast cells in the healing process remains to be elucidated.

Mast cell degranulation products induce fibroblast proliferation. When activated mast cells were cocultured with fibroblasts they were found to increase collagen synthesis and stimulate fibroblast proliferation, indicating a direct involvement of mast cells in the fibrotic process. Many

mast cell-derived mediators may potentially influence fibroblast growth and function. Histamine has been shown to stimulate fibroblast growth and collagen synthesis in vitro. Tryptase, the most abundant of the proteases found in mast cell granules, induces fibroblast proliferation, stimulates fibroblast chemotaxis and upregulates type I collagen production. Furthermore, mast cells are important sources of TGF-β, bFGF and VEGF, factors that can regulate fibroblast growth, modulate extracellular matrix metabolism and stimulate angiogenesis. Finally, mast cells may influence healing and tissue remodeling by expressing gelatinases A and B, which are implicated in extracellular matrix degradation and angiogenesis [152].

5.4. Fibroblasts and extracellular matrix remodeling

Macrophages, mast cells and lymphocytes create an environment rich in inflammatory cells, capable of regulating neovessel formation, fibroblast proliferation and extracellular matrix metabolism, through the production of a variety of cytokines and growth factors. Fibroblasts produce the extracellular matrix constituents needed to support cell ingrowth and newly formed blood vessels carry oxygen and nutrients necessary to sustain cell metabolism. Willems and colleagues [153] have previously identified and characterized the interstitial nonvascular α-smooth muscle actin (α-SMAc) positive cells, which were present in human myocardial scars 4–6 days after an infarction. These cells are phenotypically modulated fibroblasts termed myofibroblasts [154] that develop ultrastructural and phenotypic characteristics of smooth muscle cells. They are the predominant source of collagen mRNA in healing myocardial infarcts. Myofibroblasts transiently appear during granulation tissue formation and become apoptotic when the scar matures. TGF-β appears to have an important role in myofibroblast differentiation during wound healing by regulating α-SMAc expression in these cells [155]. Persistent expression of α-SMAc by fibroblasts has been described for at least 8 weeks after a nonreperfused myocardial infarct in the rat [156,157]. Cardiac myofibroblasts stain positive for vimentin, but do not express smooth muscle myosin, calponin and desmin [158]. Recent experiments have shown that myofibroblasts in the healing scar express a homologue of the Drosophila tissue polarity gene frizzled (fz2), when migrating into the granulation tissue, which may be involved in the spatial control of cardiac wound repair after infarction [159]. In addition, expression of the embryonal isoform of smooth muscle myosin heavy chain (SMemb) has been demonstrated in reperfused canine myocardial infarcts [10]. SMemb expression may reflect the dedifferentiation and phenotypic plasticity of myofibroblasts following cardiac injury, which may facilitate wound repair. Myofibroblasts are undifferentiated cells that may be capable of assuming a variety of different roles, such as extracellular matrix metabolism, neovessel formation and contractile activity [160–162].

The reparative phase of healing involves activation of proteinases, which are critical for cell migration and extracellular matrix remodeling. Recent studies have demonstrated that deficiency of urokinase-type plasminogen activator (uPA) protected mice undergoing left coronary artery ligation against myocardial rupture [163]. However, uPA -/- mice also showed impaired scar formation and infarct neovascularization. Furthermore, plasminogen-deficient mice showed a profound disturbance in healing suggesting a crucial role for the proteolytic system in regulating cardiac repair [164]. Matrix metalloproteinase (MMP) expression is upregulated in the infarcted myocardium [165,166] and may have a prominent role in extracellular matrix remodeling. Administration of MMP inhibitors [167] and targeted deletion of MMP-9 [168] attenuated left ventricular enlargement in murine myocardial infarction.

5.5. Temporal regulation of angiogenesis in the evolving and healing infarct

Formation of new blood vessels is critical for supplying the healing infarcted myocardium with oxygen and nutrients necessary to sustain metabolism. Angiogenesis is dependent on a complex interaction between extracellular matrix, endothelial cells and pericytes in response to an imbalance in the presence of angiogenic compared to angiostatic factors in the local environment [169–173]. Myocardial infarction is associated with an early release of angiogenic factors in the injured areas. Numerous investigations have indicated that VEGF, IL-8 and bFGF, all potent angiogenic agents, are rapidly induced in the ischemic myocardium [119,174,175] and may have a role in enhancing infarct neovascularization. Recently, we hypothesized that expression of angiostatic factors in the early stages of reperfusion may inhibit onset of angiogenesis until the injured myocardium has been cleared from dead cells and debris by infiltrating phagocytes and a fibrin-rich provisional matrix is formed in order to support ingrowth of new blood vessels [176]. Thus, we examined regulation of the angiostatic CXC chemokine Interferon-γ inducible Protein (IP)-10 [177–180]. IP-10 mRNA expression peaked after 1–3 h of reperfusion and was markedly decreased by 10 h of reperfusion. IP-10 mRNA and protein were localized in the venular endothelium of ischemic myocardial segments. By 24 h of reperfusion, neither IP-10 mRNA nor protein were detected. The earliest histological indication of angiogenesis began at 24 h. Isolated canine jugular vein endothelial cells expressed high levels of IP-10 and IL-8 message upon stimulation with TNF-α and endotoxin. TGF-β but not IL-10 decreased TNF-α-mediated IP-10 expression in canine endothelial cells [176]. In contrast, TNF-α-mediated IL-8 induction was not affected by incubation with IL-10 or TGF-β. We suggest that IP-10 downregulation after an early dramatic peak

following myocardial ischemia and reperfusion may allow unopposed VEGF and IL-8-mediated angiogenic activity. TGF-β may have an important indirect role in promoting angiogenesis following experimental myocardial infarction by suppressing expression of IP-10 [176].

6. Anti-inflammatory strategies following myocardial infarction. Are they doomed to fail?

The importance of the inflammatory cascade in myocardial infarction has been recognized and thoroughly investigated for the last 25 years. A vast body of evidence suggested a role for a variety of inflammatory mediators in myocardial infarction. In addition, numerous experimental studies have shown a dramatic reduction in infarct size with the use of specific anti-inflammatory strategies. However, attempts to mitigate inflammatory injury in clinical practice have, in general, been unsuccessful. The catastrophic experience of the methylprednisolone trial emphasized the need for a better understanding of the cellular and molecular events associated with the inflammatory response to achieve effective suppression of injurious processes without interfering with healing and cardiac repair. Recently, the disappointing results of the phase II anti-CD18 trials [181] led to criticism regarding the usefulness of strategies targeting the inflammatory cascade in myocardial infarction. It has been suggested that these failures may represent the inherent risk of using animal models, which may have fundamental differences from the respective human disease process. Although species-specific effects may be significant in some cases, the most important lesson we have learned from studying experimental myocardial infarction is that a sound understanding of the biology is necessary before a specific intervention is pursued on a therapeutic basis. The inflammatory cascade is based on a complex network of molecular steps mediated by molecules with pleiotropic effects, dictated by critical cellular, spatial and temporal variables. Typical properties of cytokines in networks are redundancy, pleiotropy, synergistic activity and antagonistic effects upon each other. Thus, cytokines and other inflammatory mediators, which may appear reasonable therapeutic targets considering their injurious role in the early stages of the inflammatory response may also be necessary as regulators of cardiac repair. For example, TNF-α and IL-6 may have a role in the initial inflammatory injury associated with myocardial ischemia [56,131], however they may also represent important regulators of myocyte apoptosis and cardiac repair [64,134]. We are only beginning to elucidate the role and significance of various cytokines and growth factors in healing of a myocardial infarction. Successful application of inflammation-related interventions in the treatment of myocardial infarction will require a more complete understanding of the specific molecular steps involved in the regulation of ischemic cardiac injury and repair.

Acknowledgements

The authors wish to thank Concepcion Mata and Sharon Malinowski for their editorial assistance with the manuscript. This work was supported by NIH Grant HL-42550, the DeBakey Heart Center and a grant from the Methodist Hospital Foundation.

References

[1] Entman ML, Smith CW. Postreperfusion inflammation: a model for reaction to injury in cardiovascular disease. Cardiovasc Res 1994;9:1301–1311.
[2] Frangogiannis NG, Youker KA, Rossen RD et al. Cytokines and the microcirculation in ischemia and reperfusion. J Mol Cell Cardiol 1998;12:2567–2576.
[3] Frangogiannis NG, Entman ML. Role of inflammation following myocardial ischemia and reperfusion. In: Becker RC, editor, Textbook of coronary thrombosis and thrombolysis, Dordrecht: Kluwer Academic, 1997, pp. 569–584.
[4] Mehta JL, Li DY. Inflammation in ischemic heart disease: response to tissue injury or a pathogenetic villain? Cardiovasc Res 1999;2:291–299.
[5] Bolli R. Oxygen-derived free radicals and postischemic myocardial dysfunction ('stunned myocardium'). J Am Coll Cardiol 1988;1:239–249.
[6] Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999;2:609–634.
[7] Libby P, Maroko PR, Bloor CM, Sobel BE, Braunwald E. Reduction of experimental myocardial infarct size by corticosteroid administration. J Clin Invest 1973;3:599–607.
[8] Roberts R, DeMello V, Sobel BE. Deleterious effects of methylprednisolone in patients with myocardial infarction. Circulation 1976;204–206.
[9] Kloner RA, Fishbein MC, Lew H, Maroko PR, Braunwald E. Mummification of the infarcted myocardium by high dose corticosteroids. Circulation 1978;1:56–63.
[10] Richard V, Murry CE, Reimer KA. Healing of myocardial infarcts in dogs. Effects of late reperfusion. Circulation 1995;7:1891–1901.
[11] Reimer KA, Vander Heide RS, Richard VJ. Reperfusion in acute myocardial infarction: effect of timing and modulating factors in experimental models. Am J Cardiol 1993;19:13G–21G.
[12] Jugdutt BI. Effect of reperfusion on ventricular mass, topography, and function during healing of anterior infarction. Am J Physiol 1997;3(2):H1205–H1211.
[13] Solomon A, Gersh B. The open-artery hypothesis. Annu Rev Med 1998;63–76.
[14] Frangogiannis NG, Mendoza LH, Lindsey ML et al. IL-10 is induced in the reperfused myocardium and may modulate the reaction to injury. J Immunol 2000;5:2798–2808.
[15] Maroko PR, Carpenter CB, Chiariello M et al. Reduction by cobra venom factor of myocardial necrosis after coronary artery occlusion. J Clin Invest 1978;3:661–670.
[16] Shappell SB, Taylor AA, Hughes H et al. Comparison of antioxidant and nonantioxidant lipoxygenase inhibitors on neutrophil function. Implications for pathogenesis of myocardial reperfusion injury. J Pharmacol Exp Ther 1990;2:531–538.
[17] Romson JL, Hook BG, Kunkel SL et al. Reduction of the extent of

ischemic myocardial injury by neutrophil depletion in the dog. Circulation 1983;5:1016–1023.
[18] Mullane KM, Read N, Salmon JA, Moncada S. Role of leukocytes in acute myocardial infarction in anesthetized dogs: relationship to myocardial salvage by anti-inflammatory drugs. J Pharmacol Exp Ther 1984;2:510–522.
[19] Engler RL, Dahlgren MD, Morris DD, Peterson MA, Schmidt-Schoenbein GW. Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986;H314–H323.
[20] Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR. Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. Circ Res 1984;3:277–285.
[21] Hill JH, Ward PA. The phlogistic role of C3 leukotactic fragment in myocardial infarcts of rats. J Exp Med 1971;885–890.
[22] Pinckard RN, Olson MS, Giclas PC et al. Consumption of classical complement components by heart subcellular membranes in vitro and in patients after acute myocardial infarction. J Clin Invest 1975;3:740–750.
[23] Rossen RD, Michael LH, Hawkins HK et al. Cardiolipin–protein complexes and initiation of complement activation after coronary artery occlusion. Circ Res 1994;3:546–555.
[24] Vakeva AP, Agah A, Rollins SA et al. Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: role of the terminal complement components and inhibition by anti-C5 therapy. Circulation 1998;22:2259–2267.
[25] Yasojima K, Kilgore KS, Washington RA, Lucchesi BR, McGeer PL. Complement gene expression by rabbit heart: upregulation by ischemia and reperfusion. Circ Res 1998;11:1224–1230.
[26] Dreyer WJ, Michael LH, Nguyen T et al. Kinetics of C5a release in cardiac lymph of dogs experiencing coronary artery ischemia–reperfusion injury. Circ Res 1992;6:1518–1524.
[27] Birdsall HH, Green DM, Trial J et al. Complement C5a, TGF-beta 1, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the first one to five hours after reperfusion. Circulation 1997;3:684–692.
[28] Czermak BJ, Lentsch AB, Bless NM et al. Role of complement in in vitro and in vivo lung inflammatory reactions. J Leukoc Biol 1998;1:40–48.
[29] Griselli M, Herbert J, Hutchinson WL et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. J Exp Med 1999;12:1733–1740.
[30] Weisman HF, Bartow T, Leppo MK et al. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. Science 1990;4965:146–151.
[31] Lucchesi BR, Kilgore KS. Complement inhibitors in myocardial ischemia/reperfusion injury. Immunopharmacology 1997;1–2:27–42.
[32] Kilgore KS, Friedrichs GS, Homeister JW, Lucchesi BR. The complement system in myocardial ischaemia/reperfusion injury. Cardiovasc Res 1994;4:437–444.
[33] Lefer DJ, Granger DN. Oxidative stress and cardiac disease. Am J Med 2000;4:315–323.
[34] Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia–reperfusion injury. Cardiovasc Res 2000;3:446–456.
[35] Granger DN. Role of xanthine oxidase and granulocytes in ischemia–reperfusion injury. Am J Physiol 1988;6(2):H1269–H1275.
[36] Shingu M, Nobunaga M. Chemotactic activity generated in human serum from the fifth component of complement by hydrogen peroxide. Am J Pathol 1984;2:201–206.
[37] Akgur FM, Brown MF, Zibari GB et al. Role of superoxide in hemorrhagic shock-induced P-selectin expression. Am J Physiol Heart Circ Physiol 2000;2:H791–H797.
[38] Patel KD, Zimmerman GA, Prescott SM, McEver RP, McIntyre TM. Oxygen radicals induce human endothelial cells to express GMP-140 and bind neutrophils. J Cell Biol 1991;4:749–759.
[39] Lakshminarayanan V, Drab-Weiss EA, Roebuck KA. H2O2 and tumor necrosis factor-alpha induce differential binding of the redox-responsive transcription factors AP-1 and NF-kappaB to the interleukin-8 promoter in endothelial and epithelial cells. J Biol Chem 1998;49:32670–32678.
[40] Lakshminarayanan V, Beno DW, Costa RH, Roebuck KA. Differential regulation of interleukin-8 and intercellular adhesion molecule-1 by H2O2 and tumor necrosis factor-alpha in endothelial and epithelial cells. J Biol Chem 1997;52:32910–32918.
[41] Sellak H, Franzini E, Hakim J, Pasquier C. Reactive oxygen species rapidly increase endothelial ICAM-1 ability to bind neutrophils without detectable upregulation. Blood 1994;9:2669–2677.
[42] Uraizee A, Reimer KA, Murry CE, Jennings RB. Failure of superoxide dismutase to limit size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. Circulation 1987;6:1237–1248.
[43] Gallagher KP, Buda AJ, Pace D, Gerren RA, Shlafer M. Failure of superoxide dismutase and catalase to alter size of infarction in conscious dogs after 3 hours of occlusion followed by reperfusion. Circulation 1986;5:1065–1076.
[44] Richard VJ, Murry CE, Jennings RB, Reimer KA. Therapy to reduce free radicals during early reperfusion does not limit the size of myocardial infarcts caused by 90 minutes of ischemia in dogs. Circulation 1988;2:473–480.
[45] Wang P, Chen H, Qin H et al. Overexpression of human copper, zinc-superoxide dismutase (SOD1) prevents postischemic injury. Proc Natl Acad Sci USA 1998;8:4556–4560.
[46] Chen Z, Siu B, Ho YS et al. Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. J Mol Cell Cardiol 1998;11:2281–2289.
[47] Murohara Y, Yui Y, Hattori R, Kawai C. Effects of superoxide dismutase on reperfusion arrhythmias and left ventricular function in patients undergoing thrombolysis for anterior wall acute myocardial infarction. Am J Cardiol 1991;8:765–767.
[48] Flaherty JT, Pitt B, Gruber JW et al. Recombinant human superoxide dismutase (h-SOD) fails to improve recovery of ventricular function in patients undergoing coronary angioplasty for acute myocardial infarction. Circulation 1994;5:1982–1991.
[49] Maxwell SR, Lip GY. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol 1997;2:95–117.
[50] Lenardo MJ, Baltimore D. NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. Cell 1989;2:227–229.
[51] Stancovski I, Baltimore D. NF-kappaB activation: the I kappaB kinase revealed? Cell 1997;3:299–302.
[52] Shimizu N, Yoshiyama M, Omura T et al. Activation of mitogen-activated protein kinases and activator protein-1 in myocardial infarction in rats. Cardiovasc Res 1998;1:116–124.
[53] Kupatt C, Habazettl H, Goedecke A et al. Tumor necrosis factor-alpha contributes to ischemia- and reperfusion-induced endothelial activation in isolated hearts. Circ Res 1999;4:392–400.
[54] Chandrasekar B, Freeman GL. Induction of nuclear factor kappaB and activation protein 1 in postischemic myocardium. FEBS Lett 1997;1:30–34.
[55] Morishita R, Sugimoto T, Aoki M et al. In vivo transfection of cis element 'decoy' against nuclear factor-kappaB binding site prevents myocardial infarction. Nat Med 1997;8:894–899.
[56] Frangogiannis NG, Lindsey ML, Michael LH et al. Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation 1998;7:699–710.
[57] Frangogiannis NG, Entman ML. Mast cells in myocardial ischaemia and reperfusion. In: Marone G, Liechtenstein LM, Galli SJ, editors, Mast cells and basophils in physiology, pathology and host defense, London: Academic Press, 2000, pp. 507–522.
[58] Frangogiannis NG, Burns AR, Michael LH, Entman ML. Histochemical and morphological characteristics of canine cardiac mast cells. Histochem J 1999;4:221–229.

[59] Gordon JR, Galli SJ. Mast cells as a source of both preformed and immunologically inducible TNF-alpha/cachectin. Nature 1990;274–276.
[60] Gordon JR, Burd PR, Galli SJ. Mast cells as a source of multifunctional cytokines. Immunol Today 1990;12:458–464.
[61] Michael LH, Lewis RM, Brandon TA, Entman ML. Cardiac lymph flow in conscious dogs. Am J Physiol 1979;3:H311–H317.
[62] Sack MN, Smith RM, Opie LH. Tumor necrosis factor in myocardial hypertrophy and ischaemia — an anti-apoptotic perspective. Cardiovasc Res 2000;3:688–695.
[63] Belosjorow S, Schulz R, Dorge H, Schade FU, Heusch G. Endotoxin and ischemic preconditioning: TNF-alpha concentration and myocardial infarct development in rabbits. Am J Physiol 1999;6(2):H2470–H2475.
[64] Kurrelmeyer KM, Michael LH, Baumgarten G et al. Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. Proc Natl Acad Sci USA 2000;10:5456–5461.
[65] Irwin MW, Mak S, Mann DL et al. Tissue expression and immunolocalization of tumor necrosis factor-alpha in postinfarction dysfunctional myocardium. Circulation 1999;11:1492–1498.
[66] Jacobs M, Staufenberger S, Gergs U et al. Tumor necrosis factor-alpha at acute myocardial infarction in rats and effects on cardiac fibroblasts. J Mol Cell Cardiol 1999;11:1949–1959.
[67] Jolly SR, Kane WJ, Hook BG et al. Reduction of myocardial infarct size by neutrophil depletion: effect of duration of occlusion. Am Heart J 1986;4:682–690.
[68] Litt MR, Jeremy RW, Weisman HF, Winkelstein JA, Becker LC. Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia. Evidence for neutrophil-mediated reperfusion injury. Circulation 1989;6:1816–1827.
[69] Jordan JE, Zhao ZQ, Vinten-Johansen J. The role of neutrophils in myocardial ischemia–reperfusion injury. Cardiovasc Res 1999;4:860–878.
[70] Frangogiannis NG, Youker KA, Entman ML. The role of the neutrophil in myocardial ischemia and reperfusion. EXS 1996;263–284.
[71] Hansen PR. Role of neutrophils in myocardial ischemia and reperfusion. Circulation 1995;6:1872–1885.
[72] Engler RL. Free radical and granulocyte-mediated injury during myocardial ischemia and reperfusion. Am J Cardiol 1989;10:19E–23E.
[73] Ambrosio G, Weisman HF, Mannisi JA, Becker LC. Progressive impairment of regional myocardial perfusion after initial restoration of postischemic blood flow. Circulation 1989;6:1846–1861.
[74] Ambrosio G, Tritto I. Reperfusion injury: experimental evidence and clinical implications. Am Heart J 1999;2(2):S69–S75.
[75] Smith CW. Leukocyte–endothelial cell interactions. Semin Hematol 1993;4(Suppl 4):45–53.
[76] Adams DH, Shaw S. Leukocyte–endothelial interactions and regulation of leukocyte migration. Lancet 1994;831–836.
[77] Lasky LA. Selectins: interpretors of cell-specific carbohydrate information during inflammation. Science 2000;964–969.
[78] Ebnet K, Vestweber D. Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. Histochem Cell Biol 1999;1:1–23.
[79] McEver RP, Moore KL, Cummings RD. Leukocyte trafficking mediated by selectin–carbohydrate interactions. J Biol Chem 1995;19:11025–11028.
[80] Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone Jr. MA. Identification of an inducible endothelial–leukocyte adhesion molecule. Proc Natl Acad Sci USA 1987;24:9238–9242.
[81] Montgomery KF, Osborn L, Hession C et al. Activation of endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene transcription. Proc Natl Acad Sci USA 1991;15:6523–6527.
[82] Hahne M, Jager U, Isenmann S, Hallmann R, Vestweber D. Five tumor necrosis factor-inducible cell adhesion mechanisms on the surface of mouse endothelioma cells mediate the binding of leukocytes. J Cell Biol 1993;3:655–664.
[83] Sanders WE, Wilson RW, Ballantyne CM, Beaudet AL. Molecular cloning and analysis of in vivo expression of murine P-selectin. Blood 1992;3:795–800.
[84] Bosse R, Vestweber D. Only simultaneous blocking of the L- and P-selectin completely inhibits neutrophil migration into mouse peritoneum. Eur J Immunol 1994;12:3019–3024.
[85] Jutila MA, Rott L, Berg EL, Butcher EC. Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and MAC-1. J Immunol 1989;10:3318–3324.
[86] Mulligan MS, Varani J, Dame MK et al. Role of endothelial-leukocyte adhesion molecule 1 (ELAM-1) in neutrophil-mediated lung injury in rats. J Clin Invest 1991;4:1396–1406.
[87] Arbones ML, Ord DC, Ley K et al. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. Immunity 1994;4:247–260.
[88] Ley K, Bullard DC, Arbones ML et al. Sequential contribution L- and P-selectin to leukocyte rolling in vivo. J Exp Med 1995;2:669–675.
[89] Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. Cell 1993;3:541–554.
[90] Labow MA, Norton CR, Rumberger JM et al. Characterization of E-selectin-deficient mice: demonstration of overlapping function of the endothelial selectins. Immunity 1994;8:709–720.
[91] Walcheck B, Kahn J, Fisher JM et al. Neutrophil rolling altered by inhibition of L-selectin shedding in vitro. Nature 1996;6576:720–723.
[92] Hafezi-Moghadam A, Thomas KL, Prorock AJ, Huo Y, Ley K. L-Selectin shedding regulates leukocyte recruitment. J Exp Med 2001;7:863–872.
[93] Ma XL, Weyrich AS, Lefer DJ et al. Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfused cat myocardium. Circulation 1993;649–658.
[94] Weyrich AS, Ma XL, Lefer DJ, Albertine KH, Lefer AM. *In vivo* neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. J Clin Invest 1993;2620–2629.
[95] Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999;1:181–213.
[96] Alon R, Feizi T, Yuen CT, Fuhlbrigge RC, Springer TA. Glycolipid ligands for selectins support leukocyte tethering and rolling under physiologic flow conditions. J Immunol 1995;10:5356–5366.
[97] McEver RP, Cummings RD. Role of PSGL-1 binding to selectins in leukocyte recruitment. J Clin Invest 1997;11(Suppl):S97–103.
[98] Moore KL, Patel KD, Bruehl RE et al. P-Selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. J Cell Biol 1995;4:661–671.
[99] Takada M, Nadeau KC, Shaw GD, Marquette KA, Tilney NL. The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand. J Clin Invest 1997;11:2682–2690.
[100] Hayward R, Campbell B, Shin YK, Scalia R, Lefer AM. Recombinant soluble P-selectin glycoprotein ligand-1 protects against myocardial ischemic reperfusion injury in cats. Cardiovasc Res 1999;1:65–76.
[101] Palazzo AJ, Jones SP, Anderson DC, Granger DN, Lefer DJ. Coronary endothelial P-selectin in pathogenesis of myocardial ischemia–reperfusion injury. Am J Physiol 1998;5(2):H1865–H1872.
[102] Briaud SA, Ding ZM, Michael LH et al. Leukocyte trafficking and myocardial reperfusion injury in ICAM-1/P-selectin-knockout mice. Am J Physiol Heart Circ Physiol 2001;1:H60–H67.
[103] Luscinskas FW, Lawler J. Integrins as dynamic regulators of vascular function. FASEB J 1994;929–938.
[104] Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC.

Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. J Clin Invest 1989;6:2008–2017.
[105] Smith CW. Introduction: functional polarity of motile neutrophils [comment]. Blood 2000;8:2459–2461.
[106] Rot A. Neutrophil attractant/activation protein-1 (interleukin-8) induces in vitro neutrophil migration by haptotactic mechanism. Eur J Immunol 1993;1:303–306.
[107] Furie MB, Tancinco MC, Smith CW. Monoclonal antibodies to leukocyte integrins CD11a/CD18 and CD11b/CD18 or intercellular adhesion molecule-1 inhibit chemoattractant-stimulated neutrophil transendothelial migration in vitro. Blood 1991;8:2089–2097.
[108] Ding ZM, Babensee JE, Simon SI et al. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. J Immunol 1999;9:5029–5038.
[109] Lu H, Smith CW, Perrard J et al. LFA-1 is sufficient in mediating neutrophil emigration in Mac-1-deficient mice. J Clin Invest 1997;6:1340–1350.
[110] Simpson PJ, Todd III RF, Fantone JC et al. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti-Mo1, anti-CD11b) that inhibits leukocyte adhesion. J Clin Invest 1988;2:624–629.
[111] Simpson PJ, Todd III RF, Mickelson JK et al. Sustained limitation of myocardial reperfusion injury by a monoclonal antibody that alters leukocyte function. Circulation 1990;1:226–237.
[112] Arai M, Lefer DJ, So T et al. An anti-CD18 antibody limits infarct size and preserves left ventricular function in dogs with ischemia and 48-hour reperfusion. J Am Coll Cardiol 1996;5:1278–1285.
[113] Perez RG, Arai M, Richardson C et al. Factors modifying protective effect of anti-CD18 antibodies on myocardial reperfusion injury in dogs. Am J Physiol 1996;1(2):H53–H64.
[114] Palazzo AJ, Jones SP, Girod WG et al. Myocardial ischemia–reperfusion injury in CD18- and ICAM-1-deficient mice. Am J Physiol 1998;6(2):H2300–H2307.
[115] Rollins BJ. Chemokines. Blood 1997;3:909–928.
[116] Baggiolini M. Chemokines and leukocyte traffic. Nature 1998;6676:565–568.
[117] Doenicke A, Moss J, Lorenz W, Hoernecke R. Intravenous morphine and nalbuphine increase histamine and catecholamine release without accompanying hemodynamic changes. Clin Pharmacol Ther 1995;1:81–89.
[118] Sekido N, Mukaida N, Harada A et al. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. Nature 1993;6447:654–657.
[119] Kukielka GL, Smith CW, LaRosa GJ et al. Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. J Clin Invest 1995;1:89–103.
[120] Ivey CL, Williams FM, Collins PD, Jose PJ, Williams TJ. Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit. Evidence for a role for C5a and interleukin-8. J Clin Invest 1995;6:2720–2728.
[121] Montrucchio G, Alloatti G, Camussi G. Role of platelet-activating factor in cardiovascular pathophysiology. Physiol Rev 2000;4:1669–1699.
[122] Montrucchio G, Alloatti G, Mariano F et al. Role of platelet-activating factor in polymorphonuclear neutrophil recruitment in reperfused ischemic rabbit heart. Am J Pathol 1993;2:471–480.
[123] Morgan EN, Boyle Jr EM, Yun W et al. Platelet-activating factor acetylhydrolase prevents myocardial ischemia–reperfusion injury. Circulation 1999;19(Suppl):II365–II368.
[124] Jaeschke H, Smith CW. Mechanisms of neutrophil-induced parenchymal cell injury. J Leukoc Biol 1997;6:647–653.
[125] Albelda SM, Smith CW, Ward PA. Adhesion molecules and inflammatory injury. FASEB J 1994;8:504–512.
[126] Entman ML, Youker K, Shoji T et al. Neutrophil induced oxidative injury of cardiac myocytes. A compartmented system requiring CD11b/CD18-ICAM-1 adherence. J Clin Invest 1992;4:1335–1345.
[127] Youker K, Smith CW, Anderson DC et al. Neutrophil adherence to isolated adult cardiac myocytes. Induction by cardiac lymph collected during ischemia and reperfusion. J Clin Invest 1992;2:602–609.
[128] Kukielka GL, Hawkins HK, Michael L et al. Regulation of intercellular adhesion molecule-1 (ICAM-1) in ischemic and reperfused canine myocardium. J Clin Invest 1993;3:1504–1516.
[129] Youker KA, Hawkins HK, Kukielka GL et al. Molecular evidence for induction of intracellular adhesion molecule-1 in the viable border zone associated with ischemia–reperfusion injury of the dog heart. Circulation 1994;6:2736–2746.
[130] Kukielka GL, Smith CW, Manning AM et al. Induction of interleukin-6 synthesis in the myocardium. Potential role in post-reperfusion inflammatory injury. Circulation 1995;7:1866–1875.
[131] Gwechenberger M, Mendoza LH, Youker KA et al. Cardiac myocytes produce interleukin-6 in culture and in viable border zone of reperfused infarctions. Circulation 1999;4:546–551.
[132] Finkel MS, Hoffman RA, Shen L et al. Interleukin-6 (IL-6) as a mediator of stunned myocardium. Am J Cardiol 1993;13:1231–1232.
[133] Finkel MS, Oddis CV, Jacob TD et al. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science 1992;5068:387–389.
[134] Gallucci RM, Simeonova PP, Matheson JM et al. Impaired cutaneous wound healing in interleukin-6-deficient and immunosuppressed mice. FASEB J 2000;15:2525–2531.
[135] Late Assessment of Thrombolytic Efficacy (LATE) study with alteplase 6–24 hours after onset of acute myocardial infarction. Lancet 1993;8874:759–766.
[136] Michael LH, Ballantyne CM, Zachariah JP et al. Myocardial infarction and remodeling in mice: effect of reperfusion. Am J Physiol 1999;2(2):H660–H668.
[137] Kumar AG, Ballantyne CM, Michael LH et al. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation 1997;3:693–700.
[138] Trial J, Baughn RE, Wygant JN et al. Fibronectin fragments modulate monocyte VLA-5 expression and monocyte migration. J Clin Invest 1999;4:419–430.
[139] Weihrauch D, Arras M, Zimmermann R, Schaper J. Importance of monocytes/macrophages and fibroblasts for healing of micronecroses in porcine myocardium. Mol Cell Biochem 1995;1–2:13–19.
[140] Ganz T. Macrophage function. New Horiz 1993;1:23–27.
[141] Mossmann TR. Properties and functions of interleukin-10. Adv Immunol 1994;1–22.
[142] Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest 1995;2304–2310.
[143] Yang Z, Zingarelli B, Szabo C. Crucial role of endogenous interleukin-10 production in myocardial ischemia/reperfusion injury. Circulation 2000;9:1019–1026.
[144] Cervenak L, Morbidelli L, Donati D et al. Abolished angiogenicity and tumorigenicity of burkitt lymphoma by interleukin-10. Blood 2000;7:2568–2573.
[145] Silvestre JS, Mallat Z, Duriez M et al. Antiangiogenic effect of interleukin-10 in ischemia-induced angiogenesis in mice hindlimb. Circ Res 2000;6:448–452.
[146] Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1997;4:1033–1079.
[147] Ruoss SJ, Hartmann T, Caughey GH. Mast cell tryptase is a mitogen for cultured fibroblasts. J Clin Invest 1991;2:493–499.
[148] Frangogiannis NG, Perrard JL, Mendoza LH et al. Stem cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation 1998;7:687–698.
[149] Rodewald HR, Dessing M, Dvorak AM, Galli SJ. Identification of

a committed precursor for the mast cell lineage. Science 1996;818–822.
[150] Meininger CJ, Yano H, Rottapel R et al. The c-kit receptor ligand functions as a mast cell chemoattractant. Blood 1992;958–963.
[151] Patella V, de Crescenzo G, Lamparter-Schummert B et al. Increased cardiac mast cell density and mediator release in patients with dilated cardiomyopathy. Inflamm Res 1997;S31–S32.
[152] Fang KC, Wolters PJ, Steinhoff M et al. Mast cell expression of gelatinases A and B is regulated by kit-ligand and TGF-beta. J Immunol 1999;5528–5535.
[153] Willems IE, Havenith MG, De Mey JG, Daemen MJ. The alpha-smooth muscle actin-positive cells in healing human myocardial scars. Am J Pathol 1994;4:868–875.
[154] Gabbiani G. Evolution and clinical implications of the myofibroblast concept. Cardiovasc Res 1998;3:545–548.
[155] Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 1993;1:103–111.
[156] Sun Y, Weber KT. Infarct scar: a dynamic tissue. Cardiovasc Res 2000;2:250–256.
[157] Sun Y, Weber KT. Angiotensin converting enzyme and myofibroblasts during tissue repair in the rat heart. J Mol Cell Cardiol 1996;5:851–858.
[158] Frangogiannis NG, Michael LH, Entman ML. Myofibroblasts in reperfused myocardial infarcts express the embryonic form of smooth muscle myosin heavy chain (SMemb). Cardiovasc Res 2000;89–100.
[159] Blankesteijn WM, Essers-Janssen YP, Verluyten MJ, Daemen MJ, Smits J. JF. A homologue of Drosophila tissue polarity gene frizzled is expressed in migrating myofibroblasts in the infarcted rat heart. Nat Med 1997;5:541–544.
[160] Cleutjens JP, Verluyten MJ, Smiths JF, Daemen MJ. Collagen remodeling after myocardial infarction in the rat heart. Am J Pathol 1995;2:325–338.
[161] Serini G, Gabbiani G. Mechanisms of myofibroblast activity and phenotypic modulation. Exp Cell Res 1999;2:273–283.
[162] Cleutjens JP, Blankesteijn WM, Daemen MJ, Smits JF. The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. Cardiovasc Res 1999;2:232–241.
[163] Heymans S, Luttun A, Nuyens D et al. Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. Nat Med 1999;10:1135–1142.
[164] Creemers E, Cleutjens J, Smits J et al. Disruption of the plasminogen gene in mice abolishes wound healing after myocardial infarction. Am J Pathol 2000;6:1865–1873.
[165] Cleutjens JP, Kandala JC, Guarda E, Guntaka RV, Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. J Mol Cell Cardiol 1995;6:1281–1292.
[166] Lu L, Gunja-Smith Z, Woessner JF et al. Matrix metalloproteinases and collagen ultrastructure in moderate myocardial ischemia and reperfusion in vivo. Am J Physiol Heart Circ Physiol 2000;2:H601–H609.
[167] Rohde LE, Ducharme A, Arroyo LH et al. Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. Circulation 1999;23:3063–3070.
[168] Ducharme A, Frantz S, Aikawa M et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest 2000;1:55–62.
[169] Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors and their inhibitors. Nat Med 1999;12:1359–1364.
[170] Folkman J. Angiogenesis and angiogenesis inhibition: an overview. EXS 1997;1–8.
[171] Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med 2000;4:389–395.
[172] Carmeliet P, Collen D. Molecular analysis of blood vessel formation and disease. Am J Physiol 1997;5(2):H2091–H2104.
[173] Schaper W. Angiogenesis in the adult heart. Basic Res Cardiol 1991;51–56.
[174] Lee SH, Wolf PL, Escudero R et al. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. New Engl J Med 2000;9:626–633.
[175] Li J, Brown LF, Hibberd MG et al. VEGF, flk-1, and flt-1 expression in a rat myocardial infarction model of angiogenesis. Am J Physiol 1996;5(2):H1803–H1811.
[176] Frangogiannis NG, Mendoza LH, Lewallen M et al. Induction and suppression of Interferon-Inducible Protein (IP)-10 in reperfused myocardial infarcts may regulate angiogenesis. FASEB J 2001;15:1428–1430.
[177] Frangogiannis NG, Mendoza LH, Smith CW, Michael LH, Entman ML. Induction of the synthesis of the C-X-C chemokine interferon-gamma-inducible protein-10 in experimental canine endotoxemia. Cell Tissue Res 2000;3:365–376.
[178] Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). J Exp Med 1987;4:1084–1097.
[179] Angiolillo AL, Sgadari C, Taub DD et al. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. J Exp Med 1995;1:155–162.
[180] Strieter RM, Polverini PJ, Kunkel SL et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 1995;45:27348–27357.
[181] Dove A. CD18 trials disappoint again. Nat Biotechnol 2000;8:817–818.