

Mediators of myocardial inflammation, predominantly cytokines, have for many years been implicated in the healing processes after infarction. In recent years, however, more attention has been paid to the possibility that the inflammation may result in deleterious complications for myocardial infarction. The proinflammatory cytokines may mediate myocardial dysfunction associated with myocardial infarction, severe congestive heart failure, and sepsis. A growing body of literature suggests that inflammatory mediators could play a crucial role in ischemia–reperfusion injury. Furthermore, ischemia–reperfusion not only results in the local transcriptional and translational upregulation of cytokines but also leads to tissue infiltration by inflammatory cells. These inflammatory cells are a ready source of a variety of cytokines which could be lethal for the cardiomyocytes. At the cellular level it has been shown that hypoxia causes a series of well documented changes in cardiomyocytes that includes loss of contractility, changes in lipid metabolism and subsequent irreversible cell membrane damage leading to cell death. For instance, hypoxic cardiomyocytes produce interleukin-6 (IL-6) which could contribute to the myocardial dysfunction observed in ischemia–reperfusion injury. Ischemia followed by reperfusion induces a number of other multi-potent cytokines, such as IL-1, IL-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) as well as an angiogenic cytokine/growth factor, vascular endothelial growth factor (VEGF), in the heart. Interestingly, these multi-potent cytokines (e.g. TNF- $\alpha$ ) may induce an adaptive cytoprotective response in the reperfused myocardium. In this review, we have included a number of cytokines that may contribute to ventricular dysfunction and/or to the cytoprotective and adaptive changes in the reperfused heart.

**Key words:** Heart, ischemia–reperfusion, cytokine, TNF- $\alpha$ , IL-1, IL-6, TGF- $\beta$

## Role of cytokines in myocardial ischemia and reperfusion

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## Myocardial Ischemia and Reperfusion

Coronary artery disease is one of the most common cardiac disease and it takes a heavy toll of lives in industrialized countries. Stenosis of a coronary artery leads to the myocardial ischemia which provokes tissue injury that could be reversible or irreversible depending on the severity and duration of myocardial ischemia. In the case of reversible tissue injury, structural and functional properties of the myocardium completely recover after reperfusion, whereas after irreversible injury, tissue does not recover

upon reperfusion and the myocardium becomes necrotic.<sup>1,2</sup> The occurrence of myocardial infarction during an acute coronary occlusion indicates not only the lack of oxygen and blood supply but also the disruption of defense and adaptive mechanisms of the myocardium against ischemia. Myocardial responses to a critical coronary stenosis in terms of adaptation and defense could be (1) long lasting (hours to days) contractile dysfunction in the absence of any cell death (stunned myocardium),<sup>3,4</sup> (2) tolerance towards ischemia after a short period of supply–demand mismatch (preconditioned myocardium),<sup>5,6</sup> (3) myocardial angiogenesis i.e.

growth of blood vessels and development of collateral circulation;<sup>7,8</sup> and finally, (4) atrophy of cardiac myocytes in chronic coronary artery disease.<sup>9</sup>

### Molecular Phenotype of Reperfused Myocardium

Myocardial ischemia can induce an adaptive and angiogenic process which consists of a number of sequential events including endothelial cell proliferation, migration and tissue infiltration; these processes are probably regulated by polypeptide growth factors and cytokines. Recently, we and others have reported that brief periods of ischemia leave myocardium stunned without cellular necrosis.<sup>3,4,10</sup> Stunning swine myocardium with two cycles of a 10 min left anterior descending coronary artery (LAD) occlusion followed by reperfusion increases tolerance to a subsequent longer period (60 min) of ischemia.<sup>6</sup> To get an insight into the myocardial molecular response in ischemic reperfused swine myocardium, we studied the expression pattern of a number of genes which could play a pivotal role in cytoprotection and adaptation in the heart. These were the proto-oncogenes (*c-myc*, *c-fos*, *c-jun*, and *egr-1*),<sup>11</sup> heat shock proteins (HSPs; HSP-70, HSP-27, HSP-32, HSP-8),<sup>6,9,12</sup> calcium regulatory proteins<sup>13,14</sup> and angiogenic growth factors.<sup>7,15,16</sup> Proto-oncogene products have been implicated in almost every aspect of growth control, from the binding of growth factors to cell surface receptors, to signal transduction and the regulation of transcription.<sup>11,17,18</sup> HSPs are a highly conserved group of cellular proteins which are expressed in almost every type of organism from bacteria to man.<sup>19,20</sup> HSP-70 binds to ATP and helps in translocating cytoplasmic proteins in the cells and HSP-27 migrates to the nucleus in response to stress, acts as molecular chaperone and plays an important role in signal transduction.<sup>19,21-25</sup> A number of groups, including ours, have reported that myocardial ischemia and reperfusion induce HSP-70 and HSP-27.<sup>6,9,21,25</sup> Brief periods of myocardial ischemia in rabbits also cause a rapid expression of HSP-70, which is detectable at the protein level within 2 hours.<sup>26</sup> Physiological relevance of such induced HSPs is believed to provide myocardial cytoprotection and adaptation after an ischemic episode.<sup>20,23,25,27,28</sup> Ubiquitin, another highly conserved small HSP was found to be up-regulated in the reperfused myocardium.<sup>10,29</sup> It is believed that enhanced expression of ubiquitin in response to stress is followed by its reversible conjugation with abnormal proteins destined for

degradation.<sup>29-31</sup> We believe that brief coronary occlusions—reperfusion would cause molecular damage in cardiac myocytes and such damage requires the involvement of HSPs in rescuing several vital proteins and restoring myocardial function. In contrast, irreversible myocardial damage requires rapid disposal via the ubiquitin system (ubiquitination and subsequent proteolysis) to ensure proper functioning of the surviving cells. Anti-oxidative enzyme systems (such as catalase and Mn<sup>2+</sup> SOD) have also been implicated in the adaptive mechanisms underlying myocardial ischemia—reperfusion.<sup>32</sup> Very recently, we have shown that myocardial ischemia and reperfusion lead to the induction of heme oxygenase-1 (also known as HSP-32), an enzyme that generates antioxidant biliverdin; the stimulus for its enhanced expression appears to be oxygen free radicals.<sup>12,33</sup>

### Expression of Cytokines in the Heart

#### Transforming growth factor-β1

Transforming growth factor-β1 (TGF-β1) is a 25 kDa homodimeric polypeptide differentiation factor found in platelets and most organs, including the heart.<sup>34-36</sup> By using reverse transcriptase—polymerase chain reaction (RT-PCR) and Northern blot analysis, we have shown that chronic myocardial ischemia resulted in enhanced expression of TGF-β1 mRNA.<sup>15</sup> *In situ* hybridization studies revealed that TGF-β1 specific mRNA transcripts were predominantly localized in cardiac myocytes near fibrotic tissue and not in the area of the inflammatory infiltrate.<sup>36</sup> Furthermore, immunoblot analysis with a polyclonal anti-TGF-β1 antibody showed a specific band of 25 kDa in myocardial protein extracts from normal and chronically ischemic hearts.<sup>36</sup> Immunoreactive TGF-β1 was localized in the cardiomyocytes. Purkinje cells of the conduction system were consistently stained with TGF-β1 specific antibodies<sup>15,36</sup> indicating that TGF-β1 may influence the degree of differentiation in these cells. The cellular source of TGF-β1 in the heart is controversial. Thompson *et al.*<sup>34</sup> found TGF-β1 mRNA and protein in the cardiac myocytes of the infarcted tissue, whereas Eghbali<sup>35</sup> demonstrated mRNA expression of TGF-β1 only in the nonmyocyte fraction of cardiac tissue.

Although TGF-β1 is an inhibitor of endothelial cell proliferation it has been found to be angiogenic when injected subcutaneously into newborn mice<sup>37</sup> or when applied locally in wound healing experiments.<sup>38</sup> The angiogenic

response to TGF- $\beta$ 1 application appears to be an indirect one, the primary response is the chemoattraction for monocytes which then, in turn, stimulate angiogenesis.<sup>37,39</sup> TGF- $\beta$ 1 has been shown to contribute to myocardial protection during ischemic injury;<sup>40</sup> a single bolus dose of TGF- $\beta$ 1 to a rat markedly protected the heart against reperfusion injury as assessed by loss of myocardial creatinine kinase activity and a reduction in circulating TNF- $\alpha$  levels.<sup>41</sup> Studies suggest that TGF- $\beta$ 1 might be an important molecule in cardiac embryogenesis, hypertrophy, atherogenesis, healing of myocardial infarction and in the development of coronary collaterals.<sup>34,36,39,41,42</sup> TGF- $\beta$ 1 also maintains the contractility of cultured cardiomyocytes and blocks the suppressive effects of IL-1 on their beating rate by down-regulating the expression of NO-synthase.<sup>43</sup> Thus, TGF- $\beta$ 1 could be a clinically important cytokine in the treatment of reperfusion injury and inflammatory disease in the heart.

#### Tumor necrosis factor- $\alpha$

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a 17 kDa multipotent cytokine produced mainly by activated macrophages that has been implicated in several biologic processes including inflammation, immunoregulation, and angiogenesis.<sup>44</sup> It acts directly on vascular endothelium as well as on cardiomyocytes to increase the adhesion of leukocytes during inflammation.<sup>45,46</sup> TNF- $\alpha$  is similar in many ways to TGF- $\beta$  as both polypeptides induce angiogenesis *in vivo*, promote tube formation *in vitro*, but inhibit endothelial cell proliferation; this indicates that TNF- $\alpha$  is an indirect angiogenic growth factor which may stimulate other cells to produce angiogenic factors such as VEGF.<sup>45-47</sup> Biological effects of TNF- $\alpha$  in the target cell (cardiomyocytes) are initiated by its binding to high affinity cell surface receptors.<sup>48-50</sup> TNF receptors channel signals to cytoplasm and nucleus, and thereby initiate profound alterations in the metabolic pathway and nuclear transcription.<sup>51</sup> By using RT-PCR, we detected mRNAs encoding TNF- $\alpha$  in the porcine normal as well as ischemic myocardium.<sup>15</sup> By Northern hybridization, we observed that TNF- $\alpha$  specific mRNA expression was induced in the ischemic myocardium as compared with the level found in normally perfused myocardium.<sup>15</sup> Expression of TNF- $\alpha$  and its receptors has been shown in failing human hearts indicating a pivotal role for this cytokine in pathogenesis.<sup>52,53</sup> Furthermore, the literature suggests that TNF- $\alpha$  mRNA is transcribed in the adult heart at a detectable level

and may play a role in the inflammatory processes caused by myocardial ischemia.

#### TNF- $\alpha$ induced cytoprotective mechanisms in the heart

Dysfunction of the coronary endothelium as well as injury to cardiac muscle cells are the consequences of myocardial ischemia and reperfusion. Among many factors, TNF- $\alpha$  is also released into the postischemic myocardium.<sup>41,54,55</sup> These ischemia-induced cytokines may mimic local cellular injury and may contribute to the changing molecular phenotype of the postischemic myocardium. An increase in both local expression and circulating TNF- $\alpha$  has been reported in experimental animals and in patients with myocardial ischemia and infarction.<sup>54,55</sup> Interestingly, pretreatment of rat hearts with TNF- $\alpha$  was found to be protective against ischemia and reperfusion injury.<sup>56</sup> However, in the normal heart, TNF- $\alpha$  may exert negative inotropic effects by directly altering intracellular calcium homeostasis in a concentration- and time-dependent manner.<sup>57</sup> Many studies have demonstrated that TNF- $\alpha$  induces phosphorylation of a stress protein of about 28 kDa in a number of cell types including cardiomyocytes<sup>58-60</sup> and this phosphorylation results from stimulation of a G protein-coupled signal transduction pathway involving the mitogen activated protein (MAP) kinase.<sup>61-62</sup>

Perfusion of spontaneously contracting cultures of cardiomyocytes with a high dose of TNF- $\alpha$  (10,000 units/ml) led to arrhythmias and complete cessation of spontaneous contractions followed by severe loss of myocyte inotropy.<sup>63</sup> It is known that TNF- $\alpha$  as well as interleukins (IL-2, IL-3, IL-6) induce the formation of stress proteins in cultured cardiomyocytes.<sup>23,58,64</sup> HSPs participate in cellular defense mechanisms and enable cells to survive and recover from stressful conditions.<sup>19,24,27,28</sup> It is believed that the heart has its own endogenous system(s) for protecting itself against ischemia-reperfusion injury and a number of HSPs that may act as chaperones in saving vital cellular proteins from degradation have been proposed.<sup>21-28</sup> We tested the hypothesis that TNF- $\alpha$  stimulates cytoprotective mechanisms in cardiomyocytes; such mechanisms can be examined by investigating the expression pattern of various stress protein genes. In an *in vitro* model based on cultured cardiomyocytes treated with TNF- $\alpha$  we examined gene expression of several HSPs (HSP27, HSP70 and ubiquitin).<sup>64</sup> TNF- $\alpha$  induced arrhythmia and cessation of spontaneous contractions in both a concentration- and time-dependent manner. By

using molecular biological techniques we found that steady state (ubiquitin) or undetectable mRNA levels (HSP27, HSP70) were drastically increased as compared to the untreated control cells; the effects of TNF- $\alpha$  were maximal at 6–8 h of stimulation after which the expression of these stress genes declined. By Western blot analysis we found increased multiple bands of ubiquitin–protein conjugates in TNF- $\alpha$  treated cells, but no significant change in HSP27 protein accumulation was observed until 12 h. After 24 h incubation with TNF- $\alpha$  partial cellular necrosis was detected; hence, the induced expression of cytoprotective molecules such as stress proteins (HSP27, HSP70 and ubiquitin) in response to TNF- $\alpha$  may activate protective and/or repair mechanisms in cardiomyocytes which may make them more resistant toward a subsequent challenge such as ischemia. It appears that TNF- $\alpha$ , on the one hand, mimics cellular injury in the heart and on the other it stimulates synthesis of vital proteins like HSPs and Mn<sup>2+</sup> SOD making it a very interesting and relevant cytokine for the cardiovascular system. Furthermore, the ubiquitin system could play an important role in cytosolic degradation of damaged proteins in TNF- $\alpha$  treated cardiomyocytes where HSPs may counteract the proteolytic events and preserve many vital proteins. Thus, induction of genes conferring resistance to the cytotoxic property of TNF- $\alpha$  may provide a means by which cardiomyocytes can defend themselves under pathophysiological conditions.

#### Interleukin-1

Interleukin-1 (IL-1) is a multifunctional cytokine, primarily involved in the regulation of inflammatory processes; it mediates most of the acute-phase response to infection including induction of fever.<sup>65</sup> Recent evidence indicates that IL-1 produced within tissues contributes to local inflammatory reactions.<sup>66</sup> Other biological activities of interleukin-1 include induction of fibroblast growth, ICAM-1 expression and growth and differentiation of B and T cells.<sup>67</sup> Two genes are expressed for IL-1: IL-1 $\alpha$  and IL-1 $\beta$ . Although, these genes show only 20–30% amino acid homology they were shown to bind the same high-affinity receptor.<sup>68</sup> IL-1 does not possess a typical hydrophobic signal sequence for secretion and may be processed extracellularly by limited proteolysis from a high molecular mass intracellular precursor of 33 kDa to an active 17 kDa form.<sup>69,70</sup> IL-1 is produced mainly by macrophages/monocytes, T cells, B cells, fibroblasts, keratinocytes, astrocytes and endothelial cells<sup>67</sup> and has a wide range of target cells

including cardiomyocytes and vascular smooth muscle cells. It also induces prostanoid-dependent hypotension in rabbits *in vivo*<sup>71</sup> and stimulates human smooth muscle cells to secrete interleukin-6.<sup>72</sup> In chronically ischemic myocardium where focal necrosis was documented, enhanced levels of IL- $\beta$  mRNAs were found indicating a role of this cytokine in myocardial inflammation.<sup>15</sup> Han and co-workers<sup>73</sup> have observed unchanged IL-1 $\beta$  mRNA expression in non-failing and failing human heart by RT-PCR. IL-1 $\beta$  induces macrophage-type nitric oxide synthase gene expression in cardiomyocytes leading to *de novo* synthesis of NO in the heart.<sup>74</sup> Treatment of rat hearts with IL-1 $\alpha$  resulted in improved ventricular systolic pressure and overexpression of Mn<sup>2+</sup> SOD resulting in reduced ischemia–reperfusion injury.<sup>75</sup>

#### Interleukin-6

Interleukin-6 (IL-6), a secreted single chain protein of 28 kDa, located on chromosome 7 in human and on chromosome 5 in mouse, is another pluripotent cytokine.<sup>67</sup> It is released from a variety of cell types including monocytes/macrophages, fibroblasts and endothelial cells.<sup>67,76</sup> Recently, it was shown that IL-6 is also expressed by vascular smooth muscle cells in atherosclerotic lesions of genetically hyperlipidemic rabbits.<sup>77</sup> IL-6 has a wide variety of biological functions including induction of B-cell differentiation and acute-phase response.<sup>78,79</sup> Treatment of cultured rat vascular smooth muscle cells with recombinant IL-6 resulted in an increased *c-myc* mRNA level, followed by an increase in DNA synthesis and cell number, indicating that IL-6 may play a role in the proliferation of these cells.<sup>80</sup> Human vascular smooth muscle cells express and secrete IL-6 after IL-1 stimulation or during proliferation.<sup>72</sup> We have shown that IL-6 is expressed in the porcine heart at least at the mRNA level and its expression may be regulated by ischemia–reperfusion.<sup>15</sup> Furthermore, hypoxic cardiomyocytes have been shown to produce IL-6 which could contribute to ventricular dysfunction as observed after myocardial ischemia and reperfusion.<sup>81</sup> Hirano *et al.*<sup>78</sup> reported that IL-6 was expressed in cardiac myxoma cells at much higher levels than in activated lymphocytes. There is now evidence that in patients suffering from acute myocardial infarction, IL-6 may affect the progression and the healing process of this illness, because IL-6 serum levels seem to be elevated in these patients.<sup>82</sup> Recently, it was shown that mRNAs encoding IL-6 and ICAM-1 were induced in

ischemic-reperfused myocardium and they were localized in viable myocytes adjoining the necrotic infarct suggesting a highly regulated process.<sup>83</sup>

### Cytokines – Mediators of Inflammation in Ischemia and Reperfusion

A variety of cytokines including IL-1, IL-6 and IL-8 as well as TNF- $\alpha$  have been proposed as important mediators of myocardial ischemia-reperfusion injury. For example, IL-1 was found in the circulating monocytes within hours of cardiopulmonary bypass.<sup>84</sup> Maximal amounts of IL-1 were observed 24 h after extracorporeal circulation. In another related study, an elevated level of plasma IL-6 was found in the patients following bypass surgery.<sup>85</sup> Serum levels of IL-6 have also been reported to be elevated in patients after myocardial infarction.<sup>82</sup> Our own laboratory has demonstrated induction of both IL-1 $\alpha$  and IL-8 in circulating leukocytes of human patients with peak levels at 24 h post bypass.<sup>86</sup> Intravenous administration of IL-1 or TNF- $\alpha$  was associated with cardiopulmonary dysfunction in pigs subjected to cardiopulmonary bypass.<sup>87</sup> A monoclonal antibody to TNF $\alpha$  could block or attenuate the cardiopulmonary dysfunction.<sup>88</sup>

While a growing body of evidence suggests a role of cytokines in the inflammatory response associated with myocardial ischemia and reperfusion, the mechanism of cytokine induction remains speculative. It has been suggested that ischemia and reperfusion cause the activation of platelets and leukocytes as well as the activation of complement which results in the formation of C3a/C3a-des-Arg and C5a/C5a-des-Arg.<sup>89</sup> Increased levels of C3a-des-Arg and C5a-des-Arg were documented during coronary revascularization.<sup>86</sup> These complement-derived chemotactic factors can further activate polymorphonuclear leukocytes (PMN) as well as mononuclear phagocytes which in turn, generate excessive quantities of oxygen-derived free radicals.<sup>90</sup> The production of oxygen free radicals in the ischemic reperfused myocardium is well documented<sup>91</sup> and free radicals are known inducers of endogenous pyrogens such as IL-1.<sup>92</sup> The activation and accumulation of PMNs are now thought to be due to the generation of local and systemic leukocyte chemotactic factors (LCFs).<sup>93</sup> IL-1 was initially thought to induce chemotaxis, but is now shown not to possess any chemotactic activity; the previously reported chemotactic activity of this cytokine is believed to be due to contamination with other LCFs such as IL-8. IL-8 is one of the most

powerful PMN and Tlymphocyte chemotactic factors; it can stimulate PMN adherence to endothelial cells and extracellular matrix proteins which is a manifestation of ischemic reperfusion injury.<sup>94</sup> IL-8 can also illicit a respiratory burst with the generation of oxygen free radicals and a rapid but transient increase in the free intracellular Ca<sup>2+</sup> concentration.<sup>95</sup> It is quite possible that the hydroxyl radical (OH) formation associated with ischemia-reperfusion is mediated in part through IL-8 formation. Among many potential sources of *in vivo* OH formation, PMNs represent the most significant one.<sup>96</sup> The involvement of PMN in the pathogenesis of myocardial ischemia reperfusion injury has been implicated in many previous studies. During phagocytosis, PMN release their granule contents with massive production of many toxic oxygen metabolites including oxygen free radicals. Very recently, phagocytosing PMNs were found to express high levels of IL-8 mRNA. There is no doubt that IL-8 represents the most powerful neutrophil chemotactic activity; it is also highly selective for PMNs. The newly expressed IL-8 can further stimulate the degranulation and respiratory burst of neutrophils, resulting in the formation of superoxide anions and hydrogen peroxide and thus potentiate a cascade of reactions leading to a gross inflammatory response.

Another mechanism by which cytokines may potentiate inflammatory response in the ischemic reperfused heart is by enhancing phospholipase A<sub>2</sub> activity. TNF- $\alpha$  was found to stimulate phospholipase A<sub>2</sub> and release arachidonic acid leading to the biosynthesis of cyclooxygenase products.<sup>97</sup> Activation of phospholipase A<sub>2</sub> in concert with the accumulation of arachidonic acid and production of prostaglandin endoperoxides and hydroperoxides are the salient features of myocardial ischemic reperfusion injury.<sup>98</sup> Many of the cyclooxygenase products including hydroperoxides and endoperoxides are known mediators of inflammation.

Interestingly, many of the cytokines including IL-1, IL-6 and TNF- $\alpha$  have been shown to stimulate nitric oxide (NO) production.<sup>99</sup> Reports exist to indicate that IL-1 may impair the myocardium by inhibiting intrinsic regulatory mechanisms by modulating the  $\beta$ -adrenergic control of cardiac L-type Ca<sup>2+</sup> current and that this effect is mediated by the L-arginine-nitric oxide pathway.<sup>100</sup> IL-1 can uncouple the  $\beta$ -receptor from adenylyl cyclase through a pertussis toxin-sensitive substrate such as the G<sub>i</sub> protein. The generation of NO has been suggested to play a role in the impairment of ventricular function during cytokine-mediated

inflammation.<sup>101</sup> A number of recent studies have demonstrated a role for NO in myocardial ischemic reperfusion injury,<sup>101,102</sup> and there is evidence that IL-1 activates a dexamethasone-sensitive L-Arg/NO pathway.<sup>103</sup> The finding that cytokines induce NO production<sup>102,103</sup> further supports the role for NO in the augmentation of the cytokine-mediated inflammatory response.

**Cytokines Mediate Adaptive Response in Ischemic and Reperfused Myocardium**

A number of recent studies have indicated that molecular and cellular adaptation to oxidative stress makes the heart more tolerant to ischemic reperfusion injury.<sup>104</sup> It is generally believed that such oxidative stress adaptation is mediated by the induction of several stress proteins including antioxidants and HSPs. At low doses, cytokines such as IL-1 and TNF- $\alpha$  can induce the expression of HSPs by adapting the heart to oxidative stress.<sup>59</sup> IL-1 and IL-6 can also induce antioxidant enzymes such as Mn<sup>2+</sup> SOD.<sup>105</sup> IL-1 has been found to function as a therapeutic agent when used at low doses,<sup>106</sup> and it possesses the ability to downregulate its own receptors thus explaining the protective role of IL-1 to lethal challenges by hypoxia or ischemia.<sup>107</sup>

One of the prominent features of myocardial ischemic reperfusion injury is the development

of oxidative stress. Ischemia and reperfusion potentiate the oxidative stress by at least two mechanisms. Firstly, natural antioxidative enzymes including Mn<sup>2+</sup> SOD, catalase, glutathione (GSH) peroxidase and GSH-reductase are reduced during ischemia and reperfusion.<sup>108</sup> Secondly, it is well known that oxygen-derived free radicals are produced during reperfusion of ischemic myocardium.<sup>99</sup> The results of our own study has demonstrated that pretreatment of heart with low doses of IL-1 can reduce oxidative stress and enhance several antioxidant enzymes as well as HSP-27 in the ischemic reperfused myocardium.<sup>109</sup> Such oxidative stress adaptation was associated with the reduction of ischemic reperfusion injury suggesting a strong correlation between the induction of oxidative stress inducible proteins and reduction of ischemic reperfusion injury by IL-1.

In summary, ischemia and reperfusion induce the expression of cytokines such as IL-1, IL-6, IL-8 and TNF- $\alpha$ . These cytokines presumably play a role in the inflammatory response associated with ischemic reperfusion injury. However, a small amount of IL-1 or TNF- $\alpha$  appear to induce HSPs and antioxidants that make the heart more tolerant to subsequent lethal ischemic injury. Taken together, it is tempting to speculate that ischemia-reperfusion-induced induction of cytokines reflects the adaptive response of the heart to the oxidative stress. In order to summarize

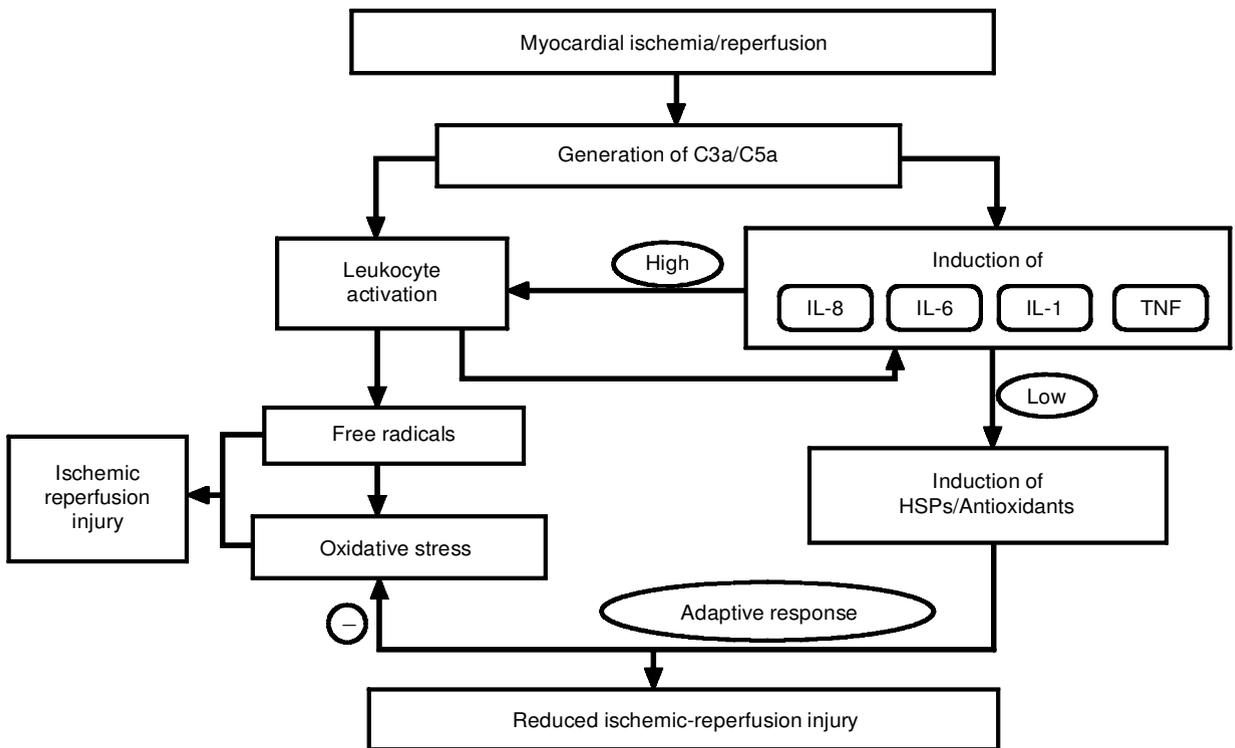


FIG. 1. A model of the role of cytokines in myocardial ischemia and reperfusion.

the existing literature in light of our own work, we propose a speculative model depicting the role of cytokines in myocardial ischemia and reperfusion (Fig. 1). We believe that in short ischemic episodes when there is a lower level of stress cytokine levels are low and the heart can enhance its own defense by inducing HSPs and antioxidants; if, however, the amount of stress is large, the induced cytokines are overwhelmed by the stress and a cascade of inflammatory reactions is initiated which leads to ischemic reperfusion injury.

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