Role of tumour necrosis factor-alpha and other cytokines in ischemia-reperfusion-induced injury in the heart

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BACKGROUND: Several investigations have implicated cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, IL-8 and transforming growth factor-beta in the pathophysiology of cellular dysfunction in ischemia-reperfusion (I/R). Although an increase in the production of these cytokines has been detected after myocardial infarction and cardiopulmonary bypass surgery, their exact role and mechanisms for inducing cardiac dysfunction are poorly understood.

OBSERVATIONS: TNF-α, transforming growth factor-beta, IL-1, IL-6 and IL-8 have frequently been studied in different cardiovascular diseases, including I/R injury in the heart. Low concentrations of TNF-α appear to exert cardioprotective effects, whereas high concentrations have been shown to produce deleterious actions in the heart. Some efforts have been made to explore the molecular mechanisms of cytokine actions; however, such information is insufficient to develop therapeutic strategies to combat their deleterious effects during the development of I/R injury in the heart.

CONCLUSIONS: In addition to a time-dependent response, the conflicting effects of cytokines seem to depend on their concentrations used in different experimental studies. It is also likely that both the beneficial and pathophysiological actions of cytokines occur concomitantly. On the basis of the existing literature, it is suggested that different ways need to be found to modify the synthesis as well as the cardiodepressant actions of cytokines to improve the therapy of ischemic heart disease.

Key Words: *Cardiac dysfunction; Cytokines; Interleukins; Ischemic heart disease; TNF-α*

Ischemia-reperfusion (I/R) injury is one of the most common cardiovascular problems and is associated with various clinical conditions such as arteriosclerosis, coronary spasm and thrombosis (1). Several investigators (2,3) have shown that accumulation of protons, cessation of oxidative metabolism and damage of electron transport are the major characteristics of myocardial ischemic injury on stoppage of blood flow. Although reinstitution of flow by procedures such as angioplasty, thrombolysis or coronary bypass surgery is essential for salvaging ischemic myocardium, re-establishment of blood flow beyond a certain period of ischemic insult has been observed to produce adverse effects commonly known as reperfusion injury or I/R injury (1,4). It has been shown that I/R injury is associated with reperfusion arrhythmias, myocardial stunning, microvascular damage and accelerated necrosis (4). Multiple factors that are involved in myocardial cell damage and cardiac dysfunction due to I/R injury show the occurrence of intracellular Ca^{2+} overload, production of oxygen-derived free radicals and alterations in different enzyme activities (5,6).

Recent studies have shown that myocardial ischemic insult promotes the formation of cytokines, a group of low molecular weight polypeptides that are autocrine contributors to cardiac dysfunction and cardiomyocyte necrosis, as well as apoptosis in I/R injury. These mediators mainly include tumour necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β) and interleukins (ILs) such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 and IL-12 (7-11). The specific targets of such mediators appear to be the endothelium and neutrophils (these deleterious mediators act on neutrophils for adherence to the vascular endothelium and, thus, induce the obstruction of capillary beds to cause a no-reflow phenomenon during reperfusion) (12,13). In fact, the accumulation of these cytokines, especially TNF-α and IL-1, within the ischemic zone damages the tissue and releases oxygen free radicals that produce myocardial cell damage and induce cardiac dysfunction (12,13). In addition, it has been reported that cytokines have numerous basic functions including activation of leukocytes, promotion of inflammation, control of cell division, induction of certain genes to produce a multitude of proteins for cellular/humoral immunity and initiation of other cytokine synthesis (14). In view of the fact that TNF-α, TGF-β, IL-1, IL-6 and IL-8 have been frequently studied in ischemic heart disease (IHD) (15,16), the present article is intended to review the role of these cytokines in I/R injury and to examine whether the modification of their synthesis is associated with alterations in cellular function.

ROLE OF TNF-α IN I/R INJURY

In the cytokine family, TNF-α is considered to be the most important mediator of cardiovascular disease. This substance was discovered by the surgeon William Coley (17), who

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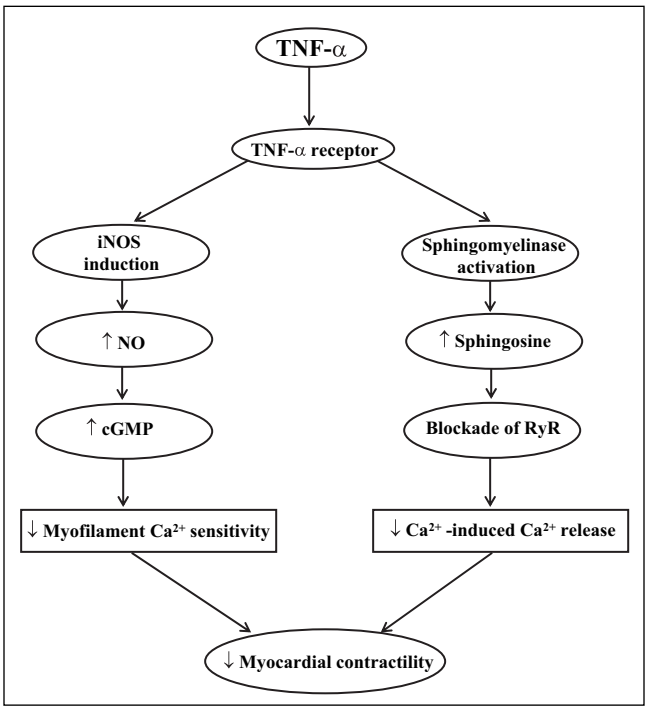

Figure 1) *Effect of tumour necrosis factor-alpha (TNF-α) on myocardial contractility. ↓ Decrease; ↑ Increase; cGMP Cyclic GMP; iNOS Inducible nitric oxide synthase; NO Nitric oxide; RyR Ryanodine receptors*

reported that TNF-α was primarily produced by lymphocytes and macrophages, and mediates endotoxin-induced tumour necrosis; other cells such as cardiomyocytes, resident cardiac macrophages and vascular smooth muscle cells have also been shown to produce TNF-α (18). TNF-α is synthesized as a 26 kDa propeptide (pro-TNF-α) in the cytosol, which is then cleaved to a 17 kDa active form by TNF-α-converting enzyme (TACE); this cleavage occurs as pro-TNF-α passes through the cell membrane. The activated form of TNF-α binds to the receptors on the cell membrane surface and triggers alterations in cytosolic protein synthesis and activation of different kinases (18,19). Similar to other protein synthesis pathways, both transcription and translational processes are involved in the regulation of TNF-α synthesis. At the transcriptional level, nuclear factor kappa B (NFκB) is the major redox-sensitive transcriptional factor associated with TNF-α production. It has been shown that inhibition of NFκB signalling completely blocks lipopolysaccharide (LPS)-induced TNF-α production (20). At the translational level, TACE plays an important role in converting pro-TNF-α to TNF-α and, thus, inhibition of TACE activity is effective in controlling the synthesis of TNF-α (21). Although LPS has been viewed as an important trigger for the production of TNF-α, several studies (22-25) have shown that TNF-α is formed and released from the rat and human myocardium after I/R injury. The pharmacological inhibition of TNF-α synthesis significantly improves the recovery of myocardial dysfunction induced by I/R (26-33). Although LPS-induced increases in TNF-α expression have been extensively studied, the mechanisms of I/R-induced TNF-α synthesis and myocardial alterations due to TNF-α during I/R in the myocardium are not fully understood.

It has been previously shown that TNF-α directly decreases contractile function in hamster, rat, dog and human myocardium (31,32,34-36). The acute negative inotropic effect of TNF-α appears to be due to alterations in Ca^{2+} handling, which are manifested by attenuated Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum and myofilament Ca^{2+} sensitivity (Figure 1). The initial contractile depression induced by TNF-α is mediated by activation of sphingomyelinase (37), which hydrolyzes the phospholipid sphingomyelin to ceramide (38). In the presence of acidic or neutral ceramidase, ceramide is deacylated to sphingosine, an endogenous second messenger (39) that causes blockade of ryanodine receptors in the sarcoplasmic reticulum and, thus, decreases Ca^{2+}-induced Ca^{2+} release and myocardial contractility (40). Delayed contractile depression by prolonged TNF-α exposure is mediated by the induction of inducible nitric oxide synthase (iNOS), with subsequent production of nitric oxide (NO). NO prevents Ca^{2+} influx via cyclic GMP-dependent inhibition of the L-type Ca^{2+} channels in sarcolemma (SL), depresses myofilament sensitivity to Ca^{2+} and, subsequently, attenuates myocardial contractility (34,41) (Figure 1). TNF-α-mediated delayed contractile depression has also been shown to be associated with desensitization of β-adrenoceptor mechanisms in the SL membrane (30). Although different investigators have revealed the involvement of both NO-dependent and NO-independent functional uncoupling of β-adrenoceptor to adenylyl cyclase (42), the exact mechanisms are not completely understood. Similarly, the cellular events in TNF-α-mediated depressed contractility after binding to the SL membrane receptors remain to be elucidated.

Different signal transduction pathways include an increase in phosphatidylcholine (PC)-specific phospholipase C (PLC) and phospholipase D (PLD) activities subsequent to the binding of TNF-α to the TNF-α receptors (TNF-Rs) (43,44). Activated PLC and PLD have been shown to hydrolyze PC to diacylglycerol (DAG) and phosphatidic acid (PA), respectively (45). DAG activates protein kinase C, which is involved in multiple signalling pathways that result in apoptosis, phosphorylation of troponin T and troponin I, and an increase in NFκB activity (46,47). Production of PA has also been associated with the pathogenesis of TNF-α-induced heart injury (10); moreover, PA has been shown to cause Ca^{2+} overload and activation of extracellular-signal-regulated protein kinase, which stimulates the activation of NFκB and, thus, plays an important role in the development of the positive feedback loop of TNF-α and NFκB (48). Furthermore, TNF-α bound to TNF-Rs triggers the activation of phospholipase A_2 and generates arachidonic acid and prostaglandins; this mechanism appears to explain the proinflammatory activities of TNF-α (49). It is important to point out that DAG, PA and arachidonic acid have also been shown to activate sphingomyelinase, the key enzyme of the sphingomyelin pathway (50), thus further aggravating TNF-α-induced contractile dysfunction.

Another mechanism of cardiac depression induced by TNF-α is associated with the induction of apoptosis in cardiomyocytes (Figure 2). It has been reported (21) that apoptosis is mainly induced via TNF-α binding to either TNF-R1 or Fas. TNF-R1 and Fas are linked with cytoplasmic proteins that are referred to as the TNF-R1-associated death domain and Fas-associated death domain, respectively. Binding of TNF-α to TNF-R1 or Fas causes conformational changes in the TNF-R1-associated and Fas-associated death domains, triggering their binding

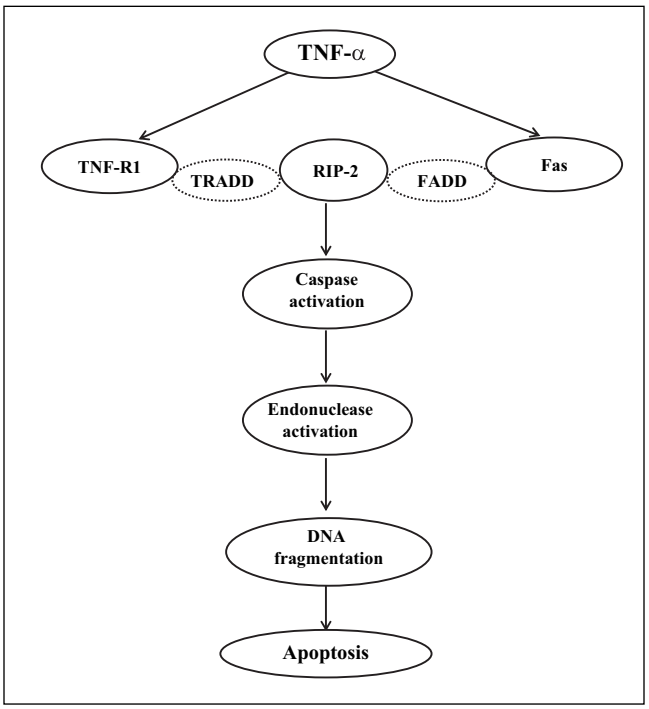

Figure 2) *Proposed outline of the pathway of tumour necrosis factor-alpha (TNF-α)-mediated apoptosis. FADD Fas-associated death domain; RIP-2 Receptor-interacting protein-2; TNF-R1 TNF-α receptor-1; TRADD TNF-R1-associated death domain*

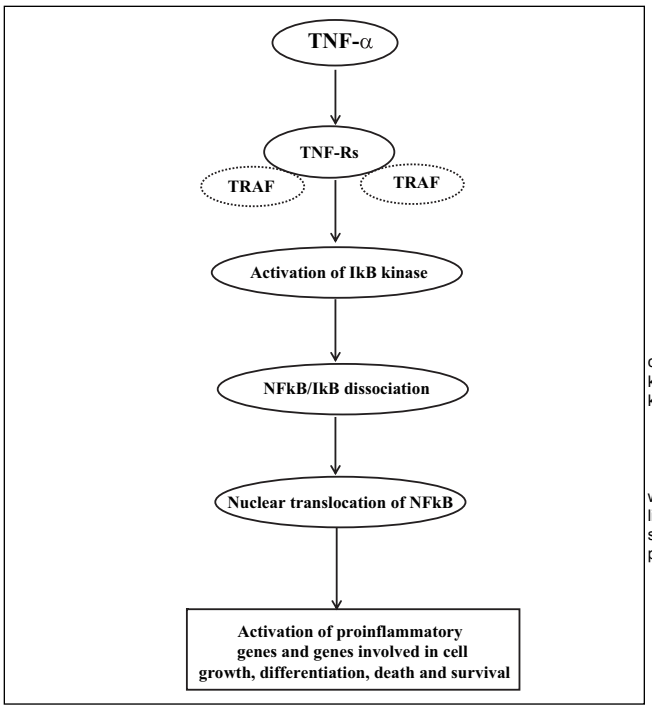

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Figure 3) *Mechanism of tumour necrosis factor-alpha (TNF-α)-mediated activation of nuclear factor kappa B (NFκB). IκB Inhibitory kappa B; TNF-Rs TNF-α receptors; TRAF TNF receptor-associated factors*

with receptor-interacting protein-2, which has a kinase domain (51). McCarthy et al (52) have shown an association of initiator caspases 1, 2 and 9 with receptor-interacting protein-2. Proteolytic cleavage of initiator caspases leads to the activation of downstream effector caspases 3, 6 and 7. Activation of effector caspases promotes the activation of endonucleases, chromatin condensation and DNA fragmentation leading to apoptosis (53). On the other hand, the binding of TNF-α to TNF-R2, which is linked with TNF-R-associated factors, causes activation of NFκB (21). The association of TNF-R1 to TNF receptor-associated factors with subsequent activation of NFκB has been previously reported (54). Activation of NFκB has been implicated in the induction of genes involved in cell proliferation, growth, survival and death (55). The proposed mechanism is outlined in Figure 3. Nonetheless, the regulatory balance between cell survival and apoptosis by NFκB activation remains to be characterized in I/R heart.

TNF-α-mediated apoptosis appears to be mediated by sphingosine and NO (56,57). Additionally, TNF-α has been indicated to be an initiator of a cytokine cascade, which results in the production of IL-6, IL-1 and IL-8, and ultimately, a worsening of the deleterious alterations induced by I/R (22,58,59). These studies seem to suggest that anti-TNF-α therapy may be valuable in I/R injury. However, it has been indicated that high circulating levels of TNF-α could be an adaptive response, which acts by promoting the shedding of TNF-Rs and reducing the number of active receptors (60). The shedding of TNF-Rs causes the formation of soluble forms of TNF-Rs (sTNF-Rs) (61). Different investigators have implicated the activation of metalloproteinase in the release of sTNF-Rs (62,63). On one hand, sTNF-Rs have been shown to exert protective effects such as inhibiting TNF-α bioactivity, serving as a TNF-α antagonist, blocking the effects of high concentrations of TNF-α and maintaining the basal TNF-α level (64). On the other hand, sTNF-Rs exert adverse effects by acting as carriers that transport TNF-α to other body compartments, by slowing the release of TNF-α and by prolonging the half-life of TNF-α by stabilizing its bioactivity (61,64). Thus, it appears that both the deleterious and beneficial effects of TNF-α may be occurring concomitantly.

A large body of evidence has accumulated to provide some information on interventions for blocking TNF-α synthesis in the ischemic heart (Table 1). p38 mitogen-activated protein kinase (MAPK) and NFκB inhibitors appear to depress the synthesis of this proinflammatory factor (21,65). Adenosine and noradrenaline, released during transient ischemia, have also been shown to reduce cardiac TNF-α production in humans (21,31,66). Maekawa et al (67) have reported reduced infarct size, decreased occurrence of arrhythmia and improved cardiac function upon subjecting TNF-α knockout mice to I/R injury compared with wild-type mice. Furthermore, TNF-α antibody, IL-1 receptor antagonist (68) and sTNF-R were also found to attenuate the deleterious effects of TNF-α in I/R heart injury in rats (33). Other interventions, including agonists of glycoprotein 130 receptor subunits (69-72), an inhibitor of TACE (73,74), an inhibitor of serine protease, aprotonin (75) and heat shock proteins (HSPs) such as HSP70 and HSP72 (76-78), have also been used in clinical and experimental trials.

In contrast to reports showing adverse effects of TNF-α, different investigators have observed that TNF-α may have protective effects during I/R (65,67,69,79-81). Lecour et al (81) have shown that TNF-α-evoked preconditioning is an effective

TABLE 1
Modification of tumour necrosis factor-alpha (TNF-α) synthesis by different pharmacological interventions in ischemic-reperfused heart

Target	Agent	Effect	References
Neutralization of TNF-α	Soluble TNF-α receptor	↓ TNF-α bioactivity	33
	IL-receptor antagonist	↓ TNF-α synthesis	68,70
NFκB antagonism	Antioxidant (vitamin E, *N*-acetylcysteine)	↓ NFκB activation	94-96
	Pentoxifylline/heparin	↓ NFκB translocation	98-100
	20S proteasome inhibitor (PS519)	↓ NFκB activation	97
	HSP70	↓ NFκB translocation	78
p38 MAPK inhibition	SB 203580	↓ TNF-α transcription	65
Metalloproteinase inhibition (TACE inhibitor)	GI 129471 Aprotonin	↓ pro-TNF-α conversion to TNF-α	73-75
Ischemic preconditioning	Adenosine	↓ TNF-α production, ↓ iNOS expression,	31,66
	Noradrenaline	↓ TNF-α bioactivity	
Induction of HSPs	HSP70, HSP72	Binding of HSPs to cytosolic TNF-α	76-78
gp130 subunit-linked agonism	IL-6 (IL-11, CT-1, leukemia inhibitory factor, oncostatin M receptor)	↓ TNF-α production	51,71,72

↓ Decrease; CT Cardiotropin; gp130 Glycoprotein 130; HSP Heat shock protein; IL Interleukin; iNOS Inducible nitric oxide synthase; MAPK Mitogen-activated protein kinase; NFκB Nuclear factor kappa B; pro-TNF-α TNF-α propeptide; TACE TNF-α-converting enzyme

intervention for the protection of I/R injury. Nelson et al (69) have indicated that pretreatment with TNF-α 24 h before the I/R period results in improved cardiac contractile function in rabbits. Several studies (79,80) have also suggested that TNF-α knockout mice may display larger infarct size after undergoing coronary ligation compared with normal mice. Such results indicate that the beneficial effects of TNF-α may be due to varying time periods, during which the heart is exposed to different concentrations of TNF-α. Although it has been indicated that the adverse and beneficial effects of TNF-α are probably dependent on the absolute levels of TNF-α during the I/R period (18), the effects of TNF-α and the mechanism of the beneficial effects remain a matter of debate. It appears that low concentrations of TNF-α exert beneficial effects, whereas high concentrations produce deleterious actions in the isolated heart (35). Similarly, in isolated cardiomyocytes, low concentrations of TNF-α cause an increase in the intracellular Ca^{2+} concentration and cardiac contraction, whereas high concentrations attenuate the electrically stimulated Ca^{2+} transient and cardiac contraction (82). Hence, to understand the pathogenesis of different heart diseases, further research is needed to elucidate the role of TNF-α in both cardioprotection and cardiodepression.

ROLE OF NFκB IN CYTOKINE PRODUCTION

NFκB is a redox-sensitive transcription factor that plays a key role in the production of most cytokines. It has been shown that NFκB exists in an inactive state in the cytoplasm of unstimulated cells because it is bound to inhibitory kappa B (IκB). The NFκB family is comprised of different subunits such as p50, p52, p65 (Rel), c-Rel, p52 and Rel B. Multiple subfamilies of IκB have also been shown to exist, including IκB-α, -β, -gamma (p105), -delta (p100), -epsilon and Bcl-3 (83). The most common active form of NFκB is the p50/p65 dimer, which is associated with the inhibitory protein IκB-α. The crucial step in the activation of NFκB is the phosphorylation of IκB by a multimeric complex referred to as IκB kinase. Anoxia, reactive oxygen species, LPS, IL-1 and TNF-α are considered to be the major stimuli that activate IκB kinase, leading to the dissociation of IκB from NFκB subunits (84). It has been reported (83,84) that multiple regulatory steps are involved in the activation of NFκB, which include nuclear translocation, phosphorylation of Rel family protein, interaction with the basal transcription complex and redox regulation. Blocking of any of the phases is likely to prevent the activation of NFκB, resulting in changes in NFκB-regulated gene expression for a broad range of physiological and pathophysiological processes. Various genes regulated by NFκB activation include cyclooxygenase-2, inhibitors of apoptotic factors, manganese superoxide dismutase, ILs (IL-1, IL-6 and IL-8), TNF-α, Fas ligands and cell adhesion molecules (83-85). In addition, the action of TNF-α on its receptors causes the activation of NFκB, which further stimulates production of TNF-α and, thus, develops a positive feedback loop (51) (Figure 4).

Recent evidence has indicated that NFκB is activated in the ischemic myocardium upon the initiation of reperfusion (86-88). Intracellular adhesion molecule-1 protein expression and iNOS are increased due to NFκB activation following I/R injury in canine heart (89). Additionally, locally produced cytokines in the myocardium have been reported to stimulate NFκB activation via PC-specific PLC and PLD pathways (48); this positive feedback loop further augments the local pathogenesis responses. Another important deleterious effect of NFκB is the promotion of apoptosis, which possibly induces irreversible myocardial damage or amplifies infarct size in myocardial infarction (90). Although Ca^{2+} is an important second messenger in the activation of NFκB in the kidneys and lymphocytes (91-93), oxidative stress is still considered an essential trigger for the activation of NFκB following I/R injury (83). An I/R-induced increase in oxidative stress causes the activation of p38 MAPK, which appears to be involved in NFκB activation followed by TNF-α production (21) (Figure 4). Several antioxidants such as *N*-acetylcysteine, α-lipoic acid and vitamin E inhibit the activation of NFκB (94-96). Cargnoni et al (94) have shown that *N*-acetylcysteine prevents the activation of NFκB by obstructing alterations in intracellular thiol,

reduced glutathione and oxidized glutathione levels. In addition, the 20S proteasome inhibitor PS-519 was found to depress the activation of NFκB due to I/R injury in the myocardium (97). In cultured vascular smooth muscle cells, HSP70, pentoxifylline, a known phosphodiesterase inhibitor, and nonanticoagulant heparin were reported to inhibit NFκB activation (77,98-100). It is important to point out that the adverse effects of NFκB during I/R injury have been determined indirectly by functional studies of NFκB-regulated genes. Therefore, further details of this pathway are needed to understand the relationship between the activation of NFκB and I/R injury.

In contrast to the negative effect of NFκB in I/R injury, some studies have reported a protective effect of NFκB activation in hearts undergoing I/R. It has been suggested that NFκB may be a key mediator of the beneficial effect of preconditioning against I/R injury (101,102), ie, ischemic preconditioning protects the heart by activating NFκB (102). It has also been suggested that NFκB stimulates the production of cytoprotective genes (eg, HSPs) (103) and NO (104). These cytoprotective genes may inhibit NFκB activation induced by the overwhelming oxidative stress during I/R and, in turn, can lead to an inhibitory effect on the production of inflammatory genes (105). Furthermore, Bach et al (106) have also suggested that NFκB mediates numerous gene expressions of proteins that inhibit cell death; in particular, these proteins include the Bcl family, zinc finger protein, endogenous antioxidants, manganese superoxide dismutase and hemeoxygenase-1. In fact, Bcl-2 has been shown to cause activation of NFκB, with a subsequent reduction in apoptosis (107). Additionally, functional NFκB signalling seems to be crucial for suppressing TNF-α-mediated apoptosis in ventricular cardiomyocytes (108). Furthermore, recent studies (109) have shown that NFκB activation prevents hypoxia-induced cell death by preserving mitochondrial function. Thus, the role of NFκB in I/R injury is controversial and the pathways mediating protective and detrimental effects still need to be elucidated. It is also important to point out that the adverse effects of NFκB during I/R injury have been indirectly suggested by functional studies of NFκB-regulated genes. Therefore, further details of this pathway are needed to understand the relationship between the activation of NFκB and I/R injury.

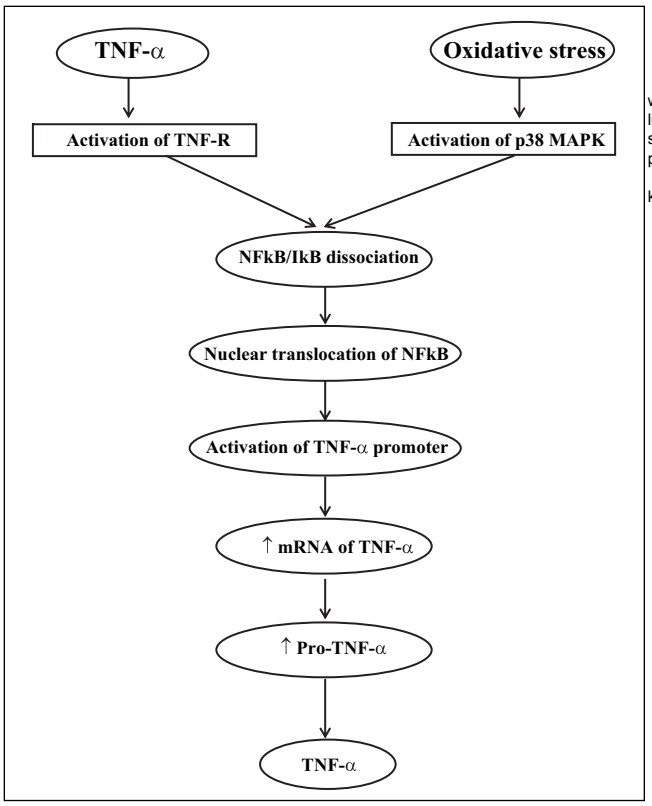

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Figure 4) *Proposed outline of the pathway of tumour necrosis factor-alpha (TNF-α) synthesis. ↑ Increase; IκB Inhibitory kappa B; MAPK Mitogen-activated protein kinase; mRNA Messenger RNA; NFκB Nuclear factor kappa B; Pro-TNF-α TNF-α propeptide; TNF-R TNF-α receptor*

ROLE OF TGF-β IN I/R INJURY

TGF is a family of peptides that exists in many mammalian tissues. Recently, TGF-β has received considerable attention for its multiple functions in controlling cell growth and responding to extracellular environmental changes (110). The most common form of TGF is TGF-β_1, which modulates various biological functions and has been identified as a powerful cardioprotective agent (111-113). Lefer et al (114) have shown that the administration of TGF-β_1 reduces infarct size in a feline model of myocardial I/R, and the protective effect seems to be due to inhibition of endothelial cell-neutrophil interactions, as well as anti-inflammatory actions that result from decreased TNF-α production. Baxter et al (115) have also observed a significant limitation of infarct size in rat heart and a reduction in apoptosis in ventricular myocytes due to TGF-β_1 administration during the early reperfusion period. It has been noted that the p42/p44 MAPK (extracellular-signal-regulated protein kinase) signalling pathway may be involved in this cardioprotective effect; its involvement is suspected because PD98059, an inhibitor of p42/p44 MAPK, abolishes the attenuated infarct size and cardiomyocyte apoptosis after TGF-β_1 treatment. In addition, the induction of the anti-apoptotic protein Bcl-2 has been implicated in the protective effect of TGF-β_1 during I/R injury in rat cardiac allografts (116). Furthermore, Mehta et al (117) have shown the participation of NO in TGF-β_1 signalling; the hypoxia-reoxygenation-induced decrease in active TGF-β_1 release was shown to be augmented by 3-morpholino-sydnonimine and nitroglycerine, known NO donors. Chen et al (118) have shown that the hypoxia-reoxygenation-mediated upregulation of iNOS expression, decrease in endothelial NOS and increase in Akt/PKB phosphorylation were attenuated by TGF-β_1 treatment, indicating the involvement of these mediators in TGF-β_1-mediated cardioprotection. Moreover, the expression of matrix metalloproteinases was also inhibited by TGF-β_1, resulting in a significant improvement in cardiac function in I/R hearts (11). Therefore, TGF-β_1 appears to promote a cardioprotective effect through a wide variety of intracellular signal pathways and, indeed, is a promising new approach to attenuate I/R injury.

ROLE OF IL-1 IN I/R INJURY

IL-1 is as an important mediator of inflammatory reactions. There are two forms of IL-1, namely, IL-α and IL-β. Because IL-β is easily detected in the blood, it has been the main focus in experimental research. Several studies (8,119,120) have shown the negative inotropic effect of IL-1 in both intact

TABLE 2
Effects of cytokines on ischemia-reperfusion-induced cardiac injury

Cytokine	Receptors	Second messengers/ pathways	Effect	References
TNF-α	TNF-α receptors	NO, sphingosine, MAPK, PKC, NFκB	Apoptosis, negative inotropic effect, initiator of synthesis of other cytokines, and inflammatory effect	27,32,34,39, 41-44,56,64
TGF-β	TGF-β_1 receptors	PKC, Bcl-2, p42/p44 MAPK	↓ Apoptosis, ↓ infarct size, ↓ endothelial cell/neutrophil interaction, anti-inflammatory, ↑ endothelium-dependent relaxation, ↓ TNF-α production	111,114-118
IL-1	IL-1β receptors	NO, MAPK, NFκB	Apoptosis, negative inotropic effect, ↓ Ca^{2+}-regulated gene expression, ↑ arrhythmogenesis	121-127
IL-6	gp130 receptors	MAPK, NO	Apoptosis, ↓ contractility, ↑ ICAM-1 production	121,130-134
IL-8	IL-8 receptors	Tyrosine kinases (Src, focal adhesion kinase)	Inflammatory effect, neutrophil migration, ↑ granule enzymatic release, ↑ oxidative burst in neutrophil	142,145,149-152

↓ Decrease; ↑ Increase; gp130 Glycoprotein 130; ICAM-1 Intracellular adhesion molecule-1; IL Interleukin; MAPK Mitogen-activated protein kinase; NFκB Nuclear factor kappa B; NO Nitric oxide; PKC Protein kinase C; TGF-β Transforming growth factor-beta; TNF-α Tumour necrosis factor-alpha

heart and isolated cardiomyocytes. IL-1 has been found to induce the expression of iNOS at both the messenger RNA (mRNA) and protein levels, and is considered to be involved in the p38 and p42/p44 MAPK signalling pathways (7). It has been observed that IL-β and TNF-α have similar pathways, causing negative inotropic effects in the myocardium (121). Furthermore, a decrease in the expression of Ca^{2+} regulatory genes has been shown to be involved in the deleterious effect of IL-1; this mechanism may be implicated in arrhythmogenesis after I/R injury (122-126). In addition, it has been observed that IL-1, like other cytokines, has the effect of inducing apoptosis in neonatal cardiac myocytes (127). Although most of the data suggest IL-1 has a primarily deleterious role in the heart, some investigations (125,128,129) have indicated a cardioprotective effect of IL-1.

ROLE OF IL-6 IN I/R INJURY

IL-6 was originally identified as a T cell-derived cytokine, but it has now been documented as a multifunctional cytokine produced by different cells types. The IL-6 family is comprised of IL-11, leukemia inhibitory factor, oncostatin M and cardiotropin-1 (CT-1). All cytokines of this family regulate intracellular signalling upon binding to receptors with a glycoprotein 130 subunit (121,130). Elevated levels of IL-6 have been detected in patients with myocardial infarction, especially during the period of reperfusion, indicating a role for IL-6 in the pathogenesis of IHD (131). Other experimental studies (132-134) have also reported an elevation of IL-6 mRNA and protein content in canine myocardium subjected to I/R injury. An increasing number of investigations (34) have shown that IL-6 serves as a direct cardiodepressant; it has been reported to inhibit myocardial contractility in hamster myocardium. It has also been shown that IL-6 reduces the peak systolic Ca^{2+} transient and contractility by increasing the production of NO and a subsequent cyclic GMP-mediated decrease in L-type Ca^{2+} channel current (135,136). Furthermore, it has been suggested that IL-6 induces the expression of iNOS in isolated cardiomyocytes, which subsequently causes a sustained depression of myocardial contractility (34,135,136). The induction of IL-6 has also been involved in the expression of intracellular adhesion molecule-1, which leads to inflammatory injury in canine ischemic heart (133,137). Conversely, pretreatment with CT-1, a member of the IL-6 family, has been shown to protect cultured cardiomyocytes against simulated ischemia/hypoxia, which may be mediated by enhancing the protein levels of HSP70 and HSP90 (138). In addition, Latchman (139) has reported that CT-1 protects the cultured cardiac cells and/or isolated rat heart from I/R injury by regulating the p42/p44 MAPK pathway. Furthermore, Craig et al (140) have shown the antiapoptotic effect of IL-6 in isolated cardiomyocytes. Therefore, further studies are needed to differentiate IL-6-mediated cell survival and cell death pathways that may be stimulated concomitantly by this cytokine.

ROLE OF IL-8 IN I/R INJURY

Like other cytokines, IL-8 is also expressed at the mRNA level in myocardium subjected to I/R injury (141). In addition, various reports (142-144) have suggested that IL-8 is important in the development of myocardial injury in human myocardial infarction. Cells such as neutrophils, monocytes, T lymphocytes and endothelial cells have also been shown to produce IL-8 (145). IL-8 is considered to be a potent participant in granule enzymatic release and oxidative burst in neutrophils, which in turn lead to further damage in the ischemic heart (121). Boyle et al (146) have shown that a depression in IL-8 level protects the heart from I/R injury in rabbits; however, the role of IL-8 in the interaction of neutrophils and endothelial cells has been controversial. Some results have shown inhibitory effects (9,147,148), whereas others have shown stimulatory effects on the migration of neutrophils through the swollen endothelium, tyrosine kinases Src and focal adhesion kinase activity (145,149-152). Although the effect of IL-8

on neutrophils is conflicting, it has been reported (121) that IL-8 is not involved in the regulation of myocardial contractility after I/R. Hence, IL-8 may be a minor factor in mediating the inflammatory effect in hearts subjected to I/R.

CONCLUSIONS

Different cytokines produced during I/R act as intracellular communication molecules within a complex network of interrelated and interacting signals, and participate in the alteration of myocardial function. Recent studies (153,154) have shown the involvement of an ischemic stimulus in cytokine production during postmyocardial infarction remodelling. As shown in Table 2, most cytokines cause deleterious activities; TNF-α, IL-1 and IL-6 cause a negative inotropic effect and induce apoptosis in myocardium subjected to I/R injury, whereas IL-8 has a mild inflammatory effect in the ischemic heart. However, some cytokines play cardioprotective roles in I/R injuries; TGF-β has been shown to promote beneficial effects by reducing cell apoptosis, decreasing infarct size and depressing TNF-α synthesis by activating multiple protein kinases and MAPK. Furthermore, CT-1, a subfamily of IL-6, has been reported to present a cardioprotective effect in I/R injury and has become an important target in cardiovascular research. From the forgoing discussion, it is also evident that cytokines such as TNF-α, IL-1 and IL-8 show conflicting results because both adverse effects and cardioprotective actions in I/R heart have been reported. It appears that the deleterious actions of these cytokines, especially TNF-α, occur with high concentrations and/or prolonged exposure, whereas the beneficial effects occur as initial responses or at low cytokine concentrations. Although both the adverse and cardioprotective effects of cytokines appear to be occurring concomitantly, the predominance of deleterious actions may play a critical role in the development of heart disease. Because interventions such as pentoxifylline have been reported to reduce the level of TNF-α and improve cardiac function in Ca^{2+} overloaded (Ca^{2+} paradox) hearts and in I/R hearts (155,156), it is suggested that pharmacological manipulation of cytokine levels may prove helpful in designing improved therapy for IHD.

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