[Frontiers in Bioscience 10, 3183-3205, September 1, 2005]

CHRONIC PERITONEAL SEPSIS: MYOCARDIAL DYSFUNCTION, ENDOTHELIN AND SIGNALING MECHANISMS

Akanksha Gupta, Sachin Brahmbhatt, Ruchita Kapoor, Lisa Loken and Avadhesh C Sharma

Cardionome Laboratory, Department of Pharmaceutical Sciences, College of Pharmacy, North Dakota State University, Fargo, ND 58105

TABLE OF CONTENTS

1. ABSTRACT

Despite advances in the understanding of pathophysiological mechanisms, there are limited pharmacotherapeutic options for sepsis, septic shock, and related pathologies. It is surprising that although sepsis-induced myocardial depression is documented in clinics, the cellular mechanisms are from clear. Alterations in molecular signaling mechanisms activated by cytokines and potent mediators such as ET-1 could pose the risk for myocardial dysfunction in sepsis. Our laboratory data suggest that the septic heart, *in vivo*, exhibits an increased time constant of left ventricular relaxation, *tau*, along with changes in LVEDP. We also observed that bigET-1-induced elevation of ET-1 correlates with cardiodynamic alterations, induction of apoptosis, and activation of p38-MAPK phosphorylation during sepsis. In light of these evidences, we emphasize that these molecular alterations in heart, both at organ and cellular level during early sepsis, need to be elucidated thoroughly.

Table 1. Pro-inflammatory and anti-inflammatory mediators in sepsis

Pro-inflammatory mediators
• TNF-alpha
• IL-1
• IL-6
• IL-8
• PAF
• Leukotrienes
• Thromboxane A2
Anti-inflammatory mediators
• IL-4
• IL-10

TNF, Tumor necrosis factor; IL, Interleukin; PAF, Plasminogen activating factor.

2. INTRODUCTION

2.1. Sepsis

Sepsis is a complex progressive immunological, metabolic, and cardiovascular disorder that results from dysregulation of normally protective anti-microbial host defense mechanisms, followed by the development of Systemic Inflammatory Response Syndrome (SIRS) in patients (1). Sepsis is a major cause of morbidity and mortality in intensive care units (ICUs), particularly in elderly, immunocompromised, and critically ill patients, worldwide (2, 3). The mortality from septic shock syndrome ranges between 20-90% depending upon the patient's age and associated pathologies (4). The incidence of sepsis has increased during last 20 years, with more than 500,000-1 million patients developing sepsis each year in the US. According to the National Vital Statistics Report (2005), septicemia and sepsis are now the 10^{th} leading cause of death as opposed to being the 13^{th} leading cause of death in 1990 in the US (5). Sepsis can cause multiple organ dysfunction, including cardiovascular complications because of the vicious cycle of inflammation and coagulation and often leads to death.

2.2. Pathophysiology

Sepsis is triggered by localized tissue infection or direct introduction of microorganisms into the bloodstream (e.g., via intravenous catheters). The septic response may also be induced by microbial exotoxins that act as superantigens (for example, toxic shock syndrome toxin). In septic shock, bacterial infection causes circulatory insufficiency when bacterial products interact with host cells and serum proteins to initiate a series of reactions that ultimately may lead to cell injury and death. These bacterial products themselves are deleterious, and the widespread and upregulated host response to these substances results in the elaboration of an extensive array of chemical mediators that lead to further cell damage. Various systems and mediators are stimulated in septic shock, including arachidonic acid metabolites (e.g., leukotrienes, prostaglandins, thromboxanes), complement system, coagulation cascade, fibrinolytic system, catecholamines, glucocorticoids, prekallikrein, bradykinin, histamines, beta-endorphins, enkephalins, adrenocorticoid hormone, circulating myocardial depressant factor(s), cachectin (tumor necrosis factor), interleukin-1, endothelin-1 (ET-1) etc. (Table 1, Figure 1). In the current review, we will discuss the role of ET-1 mediated signaling mechanisms in myocardial dysfunction during sepsis and septic shock.

2.3. Animal models of sepsis

One of the foremost challenges that the scientific community has faced in sepsis research is the availability of a clinically relevant model of sepsis. Various models of sepsis are available that replicate some signs and laboratory findings observed clinically. These models can be generally classified as infection models, intravenous infusion models, and endotoxicosis models (6). Although these models mimic several key indications as observed in clinical sepsis, the driving factor is the extent of replication of the clinical and physiological features of septic patients. Witchterman *et al* (7) have outlined the following guidelines for experimental septic models:

1. The animals should show clinical signs of sepsis such as malaise, fever, chills, generalized weakness, *etc.*
2. The septic insult should occur over a period to allow the animal to respond to the insult and attempt to overcome the challenge.
3. The model should be reproducible to allow a majority of the prepared animals to be available for the study.

2.3.1. Intravenous infusion of live bacteria and endotoxicosis model

In these models, bolus or short-term continuous infusion of large doses of bacteria (10^9-10^{10}/kg) are administered intravenously (8). The bolus intravenous infusion of live microorganisms does not simulate clinical sepsis. In clinical sepsis, the release of endotoxin into the blood stream is intermittent while the infusion of microorganisms produces an abrupt immune challenge. This model produces a rapid decrease in cardiovascular function and cardiac output with severe mortality within hours (8). There is a transient increase in serum cytokine concentrations and the magnitude is greater than that observed clinically (9). The antisepsis agents that were not clinically effective showed a positive outcome in this model. Thus, this model does not provide clinically relevant information, even when primates are used as the experimental species.

Endotoxicosis models are also widely used as they are easier to perform, and endotoxin is an important agent that induces sepsis. Models that use sublethal doses of endotoxin induce a hyperdynamic response. The continuous infusion model of endotoxin is also an advantageous model because it produces a persistent physiologic response. However, the route of administration of endotoxin is a key factor in this model. For instance, intraperitoneal endotoxin induces a hyperdynamic response in response to endotoxin. Thus, to ensure that the endotoxicosis model is clinically relevant, the physiologic variables such as mortality rate, etc. must be clearly defined (6).

2.3.2. Peritonitis model

The peritonitis models that include cecal ligation and puncture (CLP), cecal inoculum, and fecal peritonitis models have been used in both small and large animals.

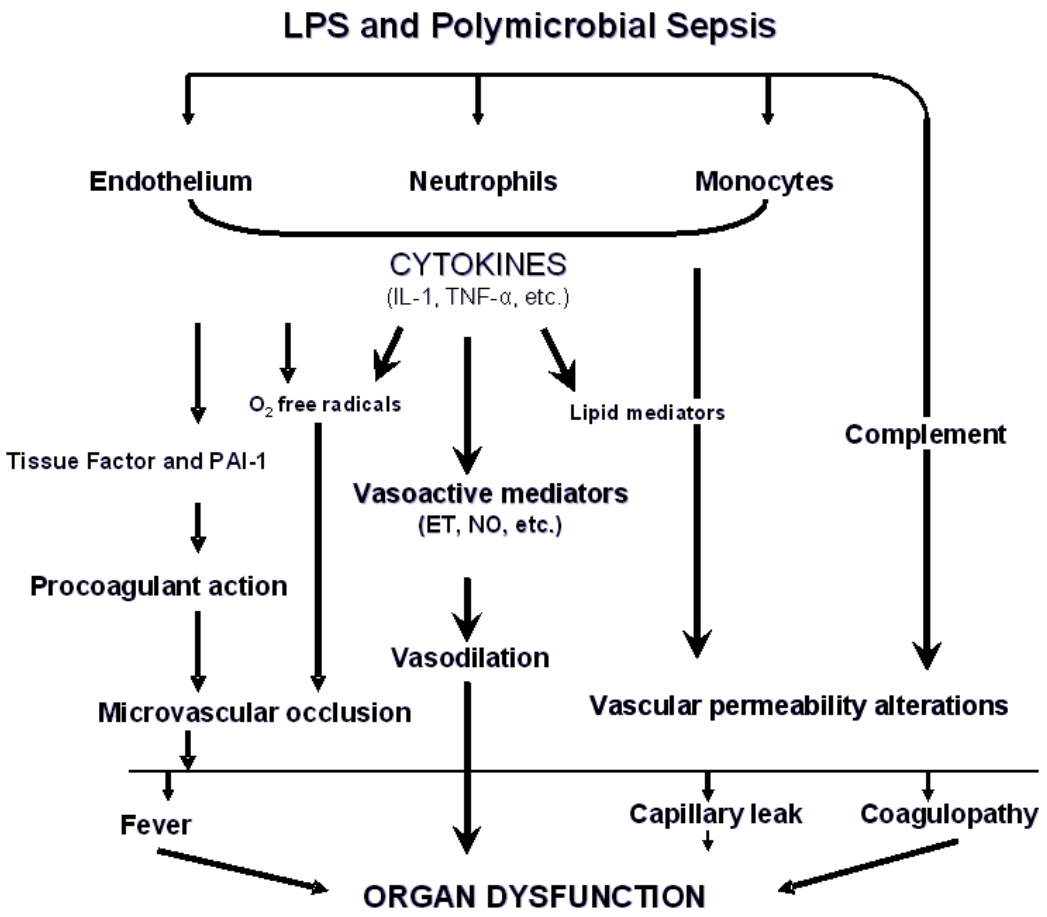

Figure 1. Pathogenesis of organ dysfunction during sepsis.

The peritonitis models induce an initial hyperdynamic cardiovascular response. The animals exhibit indications of systemic sepsis that progressively deteriorates, causing cardiovascular dysfunction and death several days after the initial insult. The serum cytokine concentrations in animals made septic using peritonitis models are similar to that observed clinically (9).

2.3.3. Fecal peritonitis

In fecal peritonitis model, bacteria suspended in a fibrin clot are implanted into the peritoneal cavity of the dog (10). However, the infectious insult in this model appears artificial and the failure to surgically treat the focus of infection in this model makes it resemble to incompletely treated peritonitis. In a study performed in male Yucatan minipigs, Kazarian *et al* (11) compared the effect of autologous fecal inoculum (FEC) and a pure culture of Escherichia coli (EC). The authors concluded from the study that i.p. EC models evoke a systemic response similar to i.v. administration of LPS or EC; however, the FEC model produced a systemic response akin to a slower developing septic process.

2.3.4. Cecal ligation and puncture (CLP) model

CLP is one of the most widely accepted and used animal model of sepsis. It satisfies the requirement of an episodic release of microorganisms into the blood stream as observed clinically. However, the model produces a rapidly lethal septic state, with mortality varying from 100% at 16-24h to 77% at five days depending on the number of punctures and size of needle used to induce sepsis. Although used widely because of clinical relevance, the CLP model also presents some disadvantages, include variation in mortality as it appears to be a personnel-dependent model.

2.3.5. Cecal inoculum model

To limit inter-personal variability and mortality in different laboratory conditions of the CLP model, cecal inoculum model was developed. The method involves an intraperitoneal administration of a quantified dose of cecal contents (40 mg/ml) obtained from a donor rat at a concentration of 200 mg/kg (12-23) (Figure 2). The cecal dose can be increased to accommodate fluid resuscitation, which greatly expands the versatility of this mode1 (24, 25). The animals made septic via the cecal inoculum method exhibit piloerection, lethargy, periocular discharge, epistaxis, lack of grooming, lose stools, etc. Evaluation of the peritoneal cavity reveals the presence of ascites and infarcts on the peritoneal organs. The animals also show leukopenia and lactic acidosis at 24 and 48-h post-sepsis induction (14). In addition, induction of sepsis by this method produces an altered profile of steroid hormones,

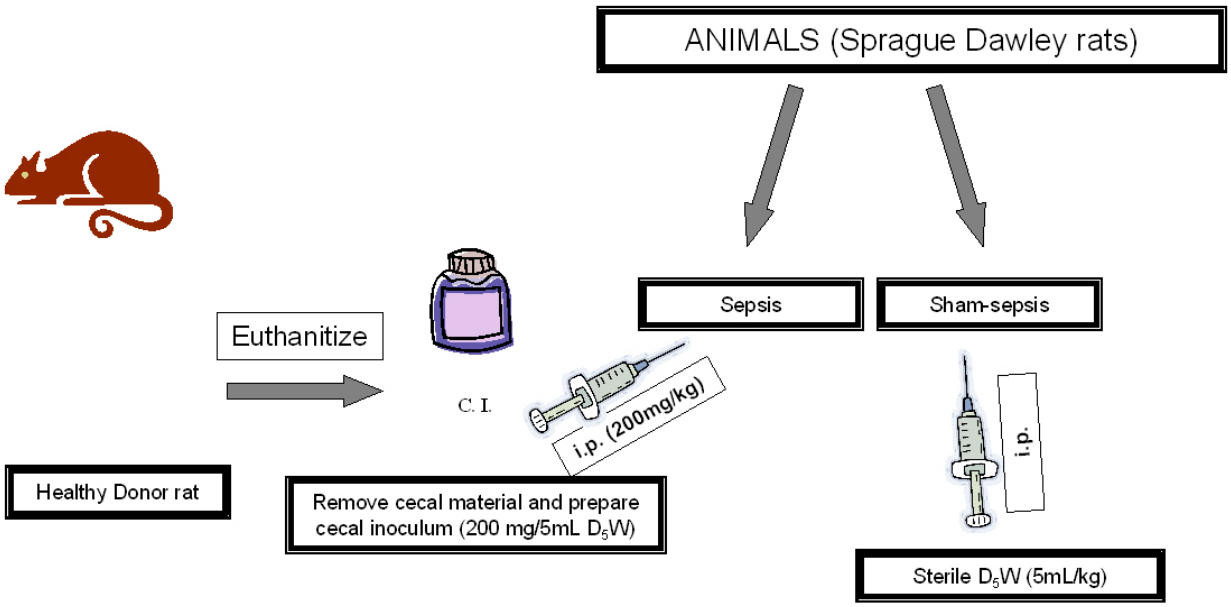

Figure 2. Induction of chronic peritoneal sepsis using cecal inoculum. Healthy donor rat is euthanitized to isolate cecal material. Cecal material is suspended in sterile 5 % dextrose water (D_5W) at a concentration of 200 mg/5 mL of D_5W to yield cecal inoculum (C.I.). Sepsis is induced in rats using intraperitoneal (i.p.) injection of C.I. using a 18 gauge needle. Animals receive 200 mg/kg of cecal inoculum i.p. After injecting the C.I., animals are gently massaged in the abdomen to evenly distribute the injectate. Sham animals receive an injection of sterile D_5W at 5 mL/kg concentration.

particularly testosterone similar to that observed in septic patients (12, 18). The mortality observed in this model generally occurs before 24h (5-15%) and between 5- and 7-days (40-60%). These septic animals also undergo a significant weight loss over a period of 48-72h post-sepsis induction and do not regain baseline weight by day 7 (14).

At 24-h post-sepsis induction, the animals are hemodynamically stable but display tachycardia. By 3-days of sepsis induction, mean arterial pressure (MAP) is similar to the pre-sepsis values, but pulse pressure remains widened. This increase in pulse pressure is attributed to a significant reduction in diastolic arterial blood pressure (13). At 7-days following sepsis induction, there is a significant reduction in MAP and pulse pressure along with elevated HR, blood lactate, and WBC count. The physiologic parameters associated with this model are described in a review (14).

3. SEPSIS-INDUCED MYOCARDIAL DYSFUNCTION

Sepsis is associated with multiple organ dysfunction, including renal dysfunction, cardiovascular dysfunction, etc. (13, 26). Myocardial dysfunction manifests in septic patients in two phases. Phase 1: Hyperdynamic stage or warm shock, and Phase 2: Hypodynamic stage or cold shock (27-29). Patients in a hypodynamic stage exhibit increased vascular tone and low cardiac output (CO) along with cold clammy skin and a thready pulse (30, 31). A hyperdynamic stage of sepsis results after adequate fluid resuscitation. Patients in this stage show peripheral vasodilatation with a high cardiac output (30) and warm dry skin with a bounding pulse. Adult septic patients generally exhibit hyperdynamic shock while the pediatric septic population may present with either hypodynamic or hyperdynamic stage of septic shock (32).

3.1. Clinical manifestations

Various studies have shown that adequately resuscitated septic patients manifest a hyperdynamic circulatory state with high CO and low systemic vascular resistance (SVR) (31). This hyperdynamic state persists until death in non-survivor septic patients. Despite strong evidence depicting a compensatory hyperdynamic phase, various studies still suggest that the septic myocardium is dysfunctional, as illustrated by decreased left ventricular stroke work index (33). The depression in the Frank-Starling curve observed in these studies can be explained by either a change in myocardial contractility or compliance (31).

Calvin *et al* (34), for the first time demonstrated myocardial dysfunction in appropriately resuscitated septic patients using portable radionuclide cineangiography (RNCA). They showed increased left ventricular end-diastolic volume index (LVEDVI) and depressed left ventricular ejection fraction (LVEF) in a subgroup of five septic patients. Similarly, Parker *et al* (35), using serial RNCA and simultaneous thermodilution CO studies on a group of 20 septic patients, demonstrated a depression in LVEF despite normal or elevated cardiac index (CI) and SVR. The non-survivors maintained normal LVEF and LVEDVI throughout the course of the illness until death. This study documented two important aspects of sepsis-

induced myocardial dysfunction. First, septic shock survivors were more likely to exhibit acute left ventricular dilation (increased LVEDVI) and decreased LVEF than non-survivors, who typically maintained normal cardiac volumes and LVEF. Second, acute changes in ventricular volumes and LVEF associated with sepsis were sustained up to 4 days, and then returned to normal in 7-10 days in survivors. This suggests that myocardial depression in human septic subjects is reversible. Doppler echocardiography revealed abnormal diastolic filling properties in left ventricles of septic patients (36, 37). Similarly, transesophageal echocardiography demonstrated isolated diastolic dysfunction and combined systolic and diastolic abnormalities (38).

In the systemic circulation, sepsis produces decreased vascular resistance and blood pressure. The left ventricle afterload is usually depressed that tends to maintain or increase CO during left ventricular contractile dysfunction (30). In contrast, right ventricular afterload is elevated due to an increase in pulmonary vascular resistance associated with lung injury and adult respiratory distress syndrome (30). Parker *et al* (39) showed a decrease in right ventricle ejection fraction (RVEF) independent of pulmonary vascular resistance and pulmonary artery pressure. They suggested that increased right ventricular afterload could not be the dominant cause of right ventricular depression in septic shock. The study also demonstrated a close temporal parallel between right and left ventricular dysfunction. However, the contribution of the right ventricle towards sepsis-induced impaired cardiac function is unknown.

3.1.1. Myocardial prognostic factors during sepsis

Three hemodynamic patterns of mortality in septic shock have been observed (40). Early mortality is due to either distributive shock (low SVR and refractory hypotension despite preserved CI) or cardiogenic shock (decreased CI). It appears that the non-survivors die due to cardiogenic shock are unable to dilate their left ventricle, as illustrated by reduced LVEDVI and CI. Patients that have increasing LVEDVI and preserved CI succumb to classic distributive shock.

The introduction of the pulmonary artery catheter (PAC) that can measure pulmonary artery wedge pressure allows for a better reflection of cardiovascular dysfunction during sepsis (41). The studies using the PAC demonstrated that adequately-volume resuscitated septic patients exhibited high CI and low SVR, including non-survivors. Hence, CI is no longer considered as a reliable predictor of mortality in sepsis (31). Presence of low SVR post-resuscitation indicated that peripheral vascular failure could be a major determinant of mortality during septic shock. Parker *et al* (40) reviewed septic patients on presentation and at 24-h to identify a prognostic criterion. They showed that, on presentation, only a heart rate (HR) < 106 bpm suggested a favorable outcome. At 24-h, HR<95 bpm, SVR index (SVRI)>1529 dynes-sec/cm^5/m^3, a reduction in HR>18 bpm and CI>0.5 L/min/m^2 predicted survival. In addition, non-survivors of septic shock show an attenuated inotropic response to a dobutamine stress test (42). Increased SVRI, venous oxygen saturation, ventricular dilation, and a reduction in diastolic blood pressure in response to dobutamine stress test predict survival in septic patients (31).

3.2. Characterization of sepsis-induced myocardial dysfunction: is *tau* the main predictor?

Several research laboratories have demonstrated that sepsis-induced myocardial dysfunction occurs at cellular level in isolated organs and clinically in humans (13, 22, 38, 43-46). Although alterations in left ventricular relaxation and contraction have been found in isolated heart preparations in various animal models of sepsis, this effect was not seen *in vivo*, primarily due to activation of compensatory changes occurring locally or centrally in peripheral vascular perfusion, venous return, pulmonary artery wedge pressure, and/or heart rate (47, 48). Myocardial dysfunction has been characterized in several animal models of sepsis.

In a canine model of CLP, systolic (reduced ejection fraction) and diastolic (reduced compliance) abnormalities of left ventricular function have been demonstrated (49). Piper *et al* (50) have demonstrated cardiac injury in isolated hearts during CLP. These septic rats exhibited 1) reduction in the rate of increase and decrease in left-ventricular pressure ($+dP/dt_{max}$ and $-dP/dt_{min}$, respectively); 2) shift in the left-ventricular Starling curves towards right and downward; and 3) reduction in the ability of the heart to develop pressure. In a study performed at 3.5 h after induction of CLP, Field *et al* (51) have shown myocardial dysfunction as reduced stroke volume and stroke work. In the same study, it has been shown that hearts isolated from CLP-induced sepsis displayed depressed peak rate of ventricular pressure development and elevated ventricular stiffness. In a canine model of *E. coli* endotoxin infusion, reduction in left ventricular filling pressure and aortic pressure along with increase in heart rate was observed (52). Abnormalities in left ventricular diastolic dysfunction have also been reported in a porcine model of endotoxemia (53). In an *E. coli* endotoxin (LPS)-induced endotoxemia model in guinea pigs, Zhong *et al* (54) reported depressed LV compliance 4-h after induction of endotoxemia. They reported that intravascular volume expansion selectively improved LV diastolic compliance of LPS hearts without affecting LV systolic function. In a similar study, Adams *et al* (55) demonstrated that the LV mechanical disadvantage of hearts treated with endotoxemia was not correlated with changes in beating frequency, active state duration, or tissue water content; neither was it surmounted by pyruvate nor by maximally effective increases in coronary flow, diastolic stretch, or extracellular Ca^{2+} concentration. In another study, Parker *et al* (39) demonstrated that LV end-diastolic pressure-volume relationships in hearts isolated from endotoxemia challenged guinea pigs were shifted upward and to the left of controls in the direction of decreased diastolic compliance. Raymond (56) reported that in a canine model of endotoxemia, hearts showed a progressive energy deficit, whereas animals surviving the experimental protocol maintained levels of ATP and creatine phosphate.

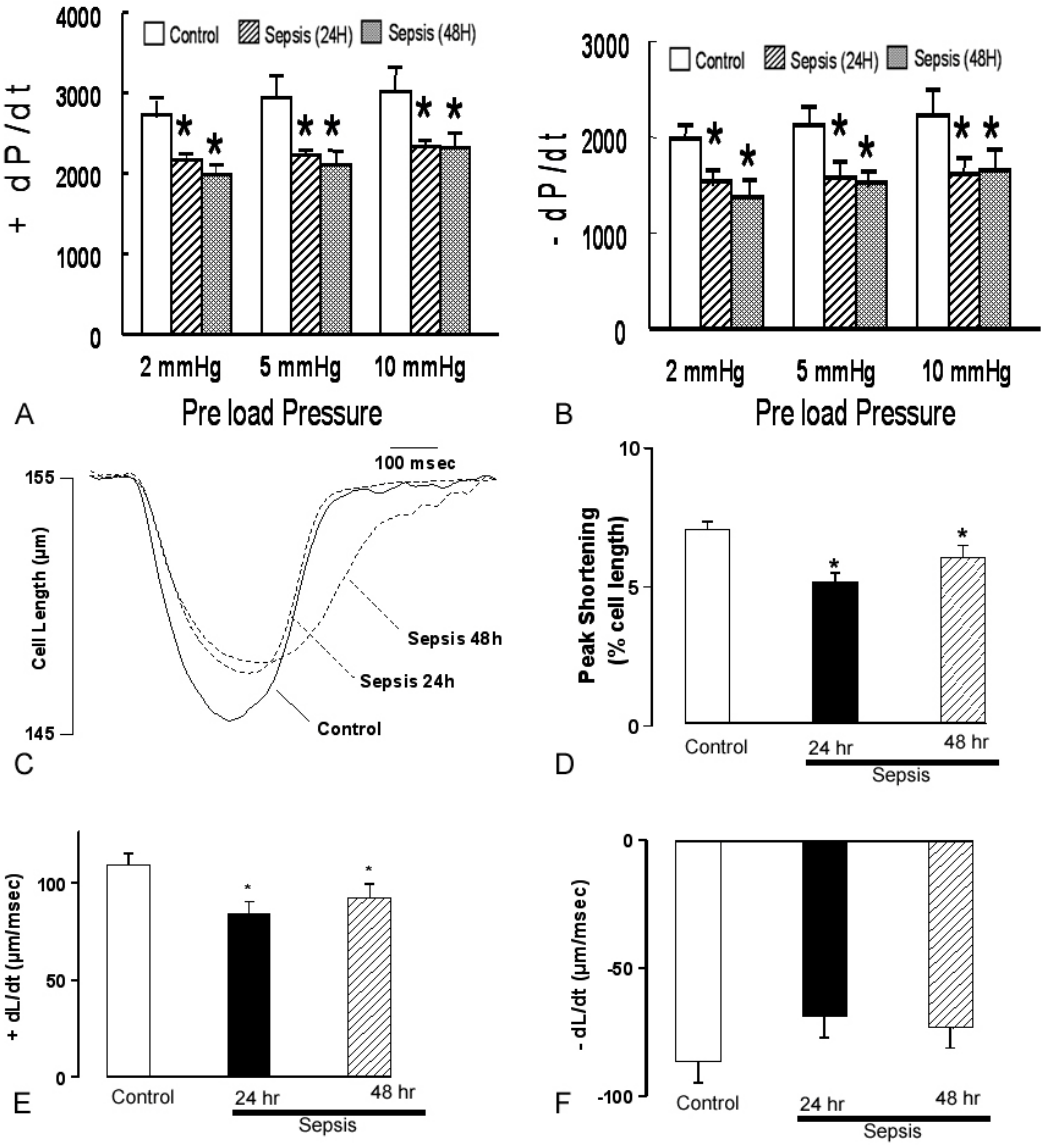

Figure 3. Rate of change of left ventricular contraction (+ dP/dt, A) & rate of change of left ventricular relaxation (–dP/dt, B) in isolated heart preparation (N=6) and contractile properties (C-F) of left ventricular myocytes isolated from control and septic (at 24 and 48-h) rats. Graphs depicted are (C) representative cell shortening traces (D) Peak shortening (E) maximal velocity of shortening (+dL/dt) (F) maximal velocity of relenghtening (-dL/dt). Data are represented as mean ± s.e.m., n= 50 per data group, * $P \leq 0.05$ as compared to control.

Similar to CLP model, in cecal inoculum model, Sharma *et al* (13) demonstrated reduced rates of left ventricular contraction and relaxation in isolated heart preparation (Figure 3A & B). In isolated ARVM, depressed contractililty (reduced peak shortening and maximal velocities of shortening and relengthening) was evident during sepsis (22) (Figure 3C-F). We observed that septic hearts were also more susceptible to a calcium-paradox mediated myocardial injury (21). In our laboratory, similar to other investigators, we did not find any change in the rates of left ventricular relaxation and contraction, *in vivo*, at 24h post-sepsis (57). However, we have demonstrated that peritoneal sepsis causes a significantly elevated left ventricular isovolumic relaxation rate constant, *tau* (57). This increase in *tau* was accompanied by an elevated left ventricular end diastolic pressure (LVEDP). However, elevation in LV isovolumic relaxation rate constant, *tau*, has been reported in response to myocardial ischemia and heart failure (58). The prolongation of *tau* occurs as a result of impaired LV relaxation (59). Patients with heart failure show increased *tau* values with a leftward shift in the LV pressure volume relationship (60). Similar to these observations in patients, in an isolated rat heart preparation from septic animals, Farias *et al* (61) reported a leftward shift in the LV pressure volume relationship showing diastolic dysfunction and altered LV diastolic compliance.

These evidences corroborate our earlier data suggesting that sepsis produced LV contractile dysfunction during early stages.

LVEDP is a parameter employed to study left ventricular filling (62-64). Sepsis-induced increased peripheral vasodilation (13) may lead to decreased stroke volume and thus decreased cardiac output. Physiologically, both at organ and vascular levels, the body compensates for the decrease in stroke volume by increasing heart rate to maintain cardiac output, leading to a hyperdynamic stage of sepsis (20). Increased heart rate in turn increases venous return and thus increases the left ventricular filling pressure as evident by increased LVEDP (48). Therefore, alterations of LVEDP during sepsis in the present study indicate progressive deterioration in myocardial function and suggest early stages of CHF. Sepsis is associated with the presence of systemic inflammatory response syndrome (SIRS). Symptoms of SIRS, including tachypnea and tachycardia, tend to increase the myocardial oxygen consumption to meet with altered metabolic state during sepsis' pathophysiology (65). Researchers have suggested that even though LV function is impaired, myocardial oxygen consumption is increased (66). Increase in LV wall stiffness causes a leftward shift in the LV pressure volume curve, causing increased LVEDP at a lower LV volume (67). LVEDP is also suggested as one of the factors responsible for increased myocardial wall stress and eventual increase in myocardial oxygen consumption (48). Results obtained in our laboratory provided evidence that sepsis and septic shock-induced alterations in *tau*, LVEDP and RPP could be the first indicators for progression of myocardial dysfunction similar to that were seen during early stages of CHF.

4. MOLECULAR MECHANISMS

4.1. Cytokines

Induction of the innate immune system during systemic infection leads to an overwhelming production of proinflammatory cytokines. Both LPS and peptidoglycans from gram-negative and gram-positive bacteria interact with TLRs to activate transcription of cytokines and pro-inflammatory mediators like TNF-alpha, IL-6, etc. Although cytokines are not directly involved in controlling vascular responses, they are known to induce the production of vasoactive mediators such as nitric oxide and ET-1.

4.1.1. Tumor necrosis factor (TNF)

TNF, a proinflammatory cytokine, is a crucial early mediator of endotoxin-induced shock (13). TNF exists as two isoforms (TNF-alpha and TNF-beta) that share similar inflammatory activities (68). TNF-alpha is predominantly derived by activated macrophages, while TNF-beta is a less potent and less abundant isoform that is produced primarily by T cells (69). Recent studies have shown that cardiac myocytes also secrete TNF-alpha in response to sepsis (70). An increased concentration of TNF-alpha has been shown to correlate with deficits in cardiac contraction and relaxation (71). In sepsis, the release of TNF-alpha in turn activates a second level of inflammatory cascades, including cytokines such as IL-6 and IL-8, lipid mediators, and reactive oxygen species, as well as up-regulating cell adhesion molecules (ICAM) that result in the initiation of inflammatory cell migration into tissues (72). TNF-alpha is detected within 20 minutes after an immune challenge (73). The concentration of TNF-alpha peaks between 90 minutes and 2-h after endotoxin injection with transient release that varies in a wide range of concentrations (from few to thousands pg/ml) (74). TNF-alpha exists as a 17-kDa molecule and has a half-life of ~30 minutes and can be measured in both plasma and serum using either immunoreactive or cytotoxic assays (64). TNF-alpha is degraded in the liver, gastro-intestinal tract (GIT), and kidneys (73). TNF-alpha acts via two receptors designated as TNFR1 (p55) and TNFR2 (p75) (75). The concentration of soluble receptors for TNF-alpha is elevated during sepsis, particularly in patients with end-organ failure (76). However, studies using monoclonal antibodies directed against TNF-alpha or soluble TNF-alpha receptors failed to improve survival in septic patients (77-79).

4.1.2. Interleukins (ILs)

Interleukin-1 (IL-1) plays a central role in the systemic immune response and is implicated during sepsis and septic shock. IL-1, a 26-kDa protein is synthesized by monocytes, macrophages, and neutrophils in response to TNF-alpha. IL-1 has been shown to exert a negative inotropic effect and depresses cardiac contractility by stimulating nitric oxide synthase (80). Gene transcription of IL-1 is accompanied by a temporal delayed transcription of IL-1 receptor antagonist (IL-ra) that functions as an endogenous inhibitor of IL-1. Recombinant IL-ra has been evaluated in three multicentre trials that yielded a reduction in mortality by 4.9% in septic patients (81, 82). However, the clinical development of this protein as a therapy has been curtailed.

Another proinflammatory cytokine, interleukin-6 (IL-6) has been implicated in the pathogenesis of sepsis. IL-6 is produced by a variety of cells, including monocytes/macrophages, endothelial cells, fibroblasts, and smooth muscle cells in response to stimulation by LPS, TNF-alpha, and IL-1. IL-6 is considered a more consistent predictor of sepsis due to its prolonged elevation in the circulation than TNF-alpha (83).

4.2. Endothelin (ET)

4.2.1. ET-1 biology

ET mechanisms have been shown to play a very important role in the pathogenesis sepsis (84). It has been reported that among all the pathophysiologic conditions possibly involving the endothelial system, sepsis is associated with the highest levels of plasma ET-1 (85). Endotoxins increase plasma ET-1 levels, along with increased mRNA expression of preproET-1 in the lungs and heart (86, 87). Experiments involving infusion of ET-1 show signs of cardiovascular complications generally associated with septic shock (88, 89), thus suggesting that ET mechanisms may be the major contributive factor to the dysfunction of vital organs during sepsis (90, 91). These major findings suggest the importance of ET mechanisms

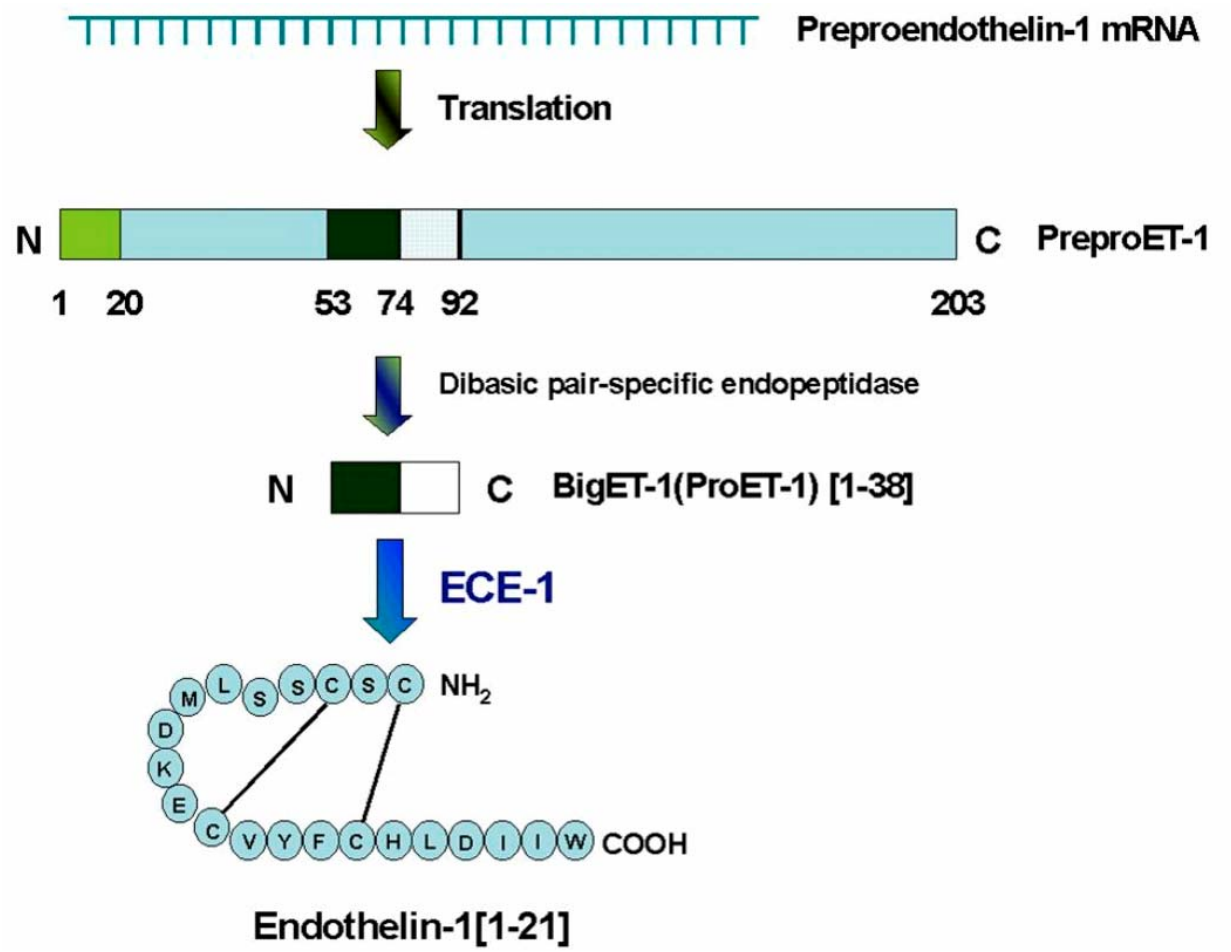

Figure 4. Biosynthetic pathway of ET-1.

in sepsis. However, the role of ET-1 in the pathogenesis of sepsis-induced myocardial dysfunction is not yet defined.

The discovery of a peptide factor EDCF (endothelium-derived contracting factor) that exhibited vasoconstrictive property (92) stirred a major interest in the scientific community worldwide in 1985. This factor was characterized in a conditioned medium of cultured bovine endothelial cells. Yanagisawa *et al* (93) isolated, purified, sequenced, and cloned this EDCF from the supernatant of cultured porcine aortic endothelial cells and named it ET. This peptide is one of the most potent vasoconstrictors with a long-lasting action. It consists of 21 amino acids with a molecular weight of 2492 Daltons. This peptide with a free amino and carboxy terminal has four cysteine residues that form two intramolecular disulfide bonds (Cys^1-Cys^5; Cys^3-Cys^{11}).

The ET family consists of three isopeptides: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). All three isopeptides are encoded by separate genes (94) but are localized differently. In humans, ET-1, ET-2, and ET-3 have been mapped to chromosome 6 [6p24-p23], chromosome 1 [1p34], and chromosome 20 [20q13.2-q13.3] (95, 96) respectively. All the isoforms have vasoconstrictor action but at varied potency. ET-2 has vasoconstriction action similar to ET-1. ET-3, however, has reduced vasoconstrictor property in comparison to the other two isoforms (94). ETs have structural similarity to the sarafotoxins, which are cardiotoxic peptides isolated from the venom of *Atractaspis engaddensis* (94).

ET is produced by a multifarious group of cells and tissues. However, the expression of individual isoforms is tissue specific. Endothelial cells predominantly generate ET-1. ET-1 is also produced by cardiac myocytes, kidney, central nervous system, and human aortic smooth muscle cells (94). ET-2 is primarily produced within the kidney and intestine (97). Myocardium, placenta, uterus, and endothelial cells also generate ET-2 but at a lower concentration (97). ET-3 is predominately produced by the brain (98). The human ET-1 gene is composed of five exons, four introns, and 5' and 3'-flanking regions distributed over 6,836 bp. Each of the five exons encodes a portion of preproET-1: exon 1 encodes the 5' untranslated region and first 22 amino acids of the precursor; exon 2 encodes ET-1, first four residues of C-terminal of bigET-1 and the Trp-Val cleavage site; exon 3 encodes the coding region of remaining bigET-1; and the "ET-like peptide" of preproET-1; exon-5 encodes the C-terminal of bigET-1 and the 3' untranslated region.

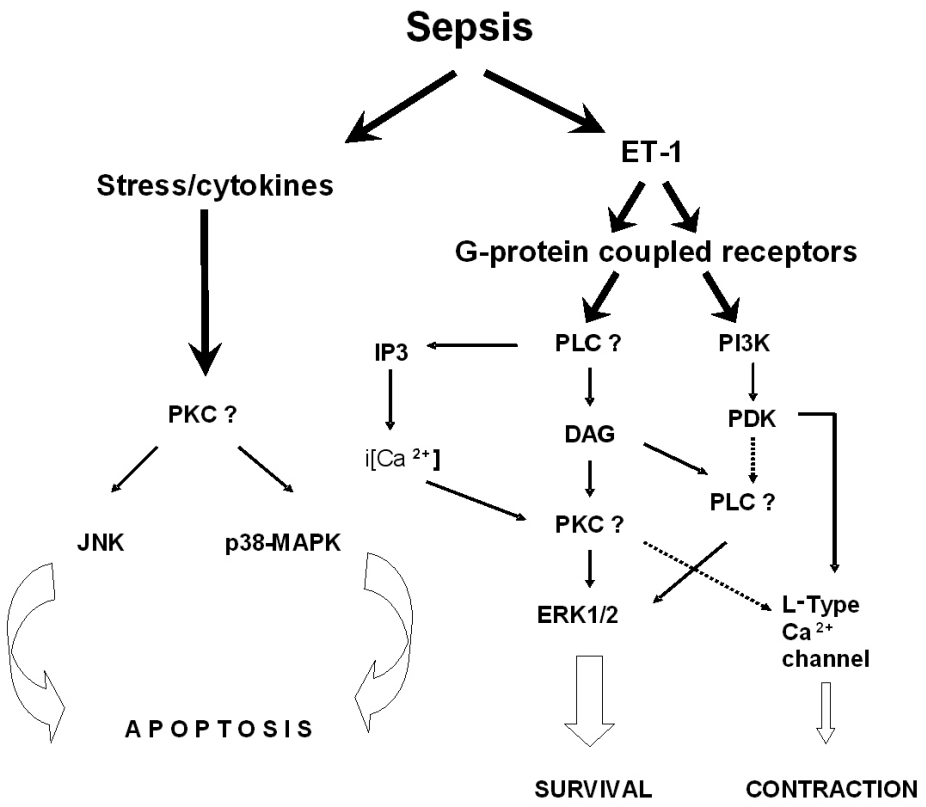

Figure 5. Sepsis-induced regulation of ET-1 and G-protein coupled receptor signaling in adult rat ventricular myocytes. Stress/cytokines mediated cascades at cellular and vascular level. It is still not known if ET-1 mediated chronic effects are mediated via activation of PKC-induced p38-MAPK/JNK phosphorylation.

ETs are synthesized from the precursor preproendothelin-1, which is a 212 amino acid peptide (99). After the signal peptide is removed, this propeptide is cleaved by a dibasic amino acid endopeptidase(s) at Arg^{52}-Cys^{53} and at Arg^{92}- Ala^{93} followed by a C-terminal trimming resulting in the formation of a 38-amino acid residue intermediate peptide, termed bigET or proET. The protease involved in the formation of the bigET fragment is a furin-like enzyme. The bigETs are subsequently cleaved at Trp^{21}-Val^{22} of ET-1 and ET-2 or at the Trp^{21}-Ile^{22} of ET-3 to form the mature peptide (Figure 4) and an inactive C-terminal (22-38) fragment. The final step in the processing of ET is carried out by a membrane-bound zinc metalloprotease, termed ET converting enzyme, ECE-1 (100). The biological significance of this enzyme stems from the fact that the molar potency of bigET was 140 times lower as compared to the mature peptide (101). It is established that preproET-1 does not possess any vasoconstrictor activity (94). The above findings suggest that the conversion of bigET-1 to the mature peptide by the endopeptidase ECE is critical for the marked vasoconstrictor activity of ET-1. Mammalian ET isopeptides mediate their pharmacological actions via binding to their receptors. These receptors belong to the guanine nucleotide-binding protein-coupled receptors (GPCRs) superfamily. Three receptor subtypes for endothelin receptors, ET_A, ET_B, and ET_C, have been identified and cloned (100-104). The ET_A receptor is predominantly expressed in cardiac myocytes but not in endothelial cells (105-107) and primarily mediates the vasoconstrictor action of endothelins. ET_B receptors are mostly expressed in endothelial cells and in vascular smooth muscle cells. ET_C receptor is identified, cloned and characterized from *Xenopus laevis* (104) but not from humans.

4.2.2. ET-1-induced signal transduction

Binding of ET-1 to its receptors induces G-protein coupled stimulation of phospholipase C. The ET_A receptor may couple to $G_{i/o}$ or G_q subtypes of G-proteins. G_q consists of an α–subunit and a member of β and γ–subunit family. The inactive G_q heterotrimer has the αq ligated to GDP. The activation of GPCRs on binding by ET-1 or an agonist stimulates exchange of GDP for GTP on αq, causing the heterotrimer to dissociate into αq (GTP) and β γ dimers (108). This dissociation leads to the activation of phosphoinositide-specific phospholipase Cβ (PI-PLCβ) (109). The PI-PLCβ hydrolyses the membrane phospholipids, phosphatidylinositol 4', 5'-biphosphate into two "second messengers": diacylglycerol (DAG) and inositol 1', 4', 5'-triphosphate [IP_3]. These two second messengers have been detected within seconds of exposure of myocytes to ET-1 (110) (Figure 5). Activation of PI-PLCβ is terminated by the innate activity of the αq (GTP) subunits. This innate activity is itself stimulated by the GTPase activating proteins (GAPs) (111). IP_3 diffuses into

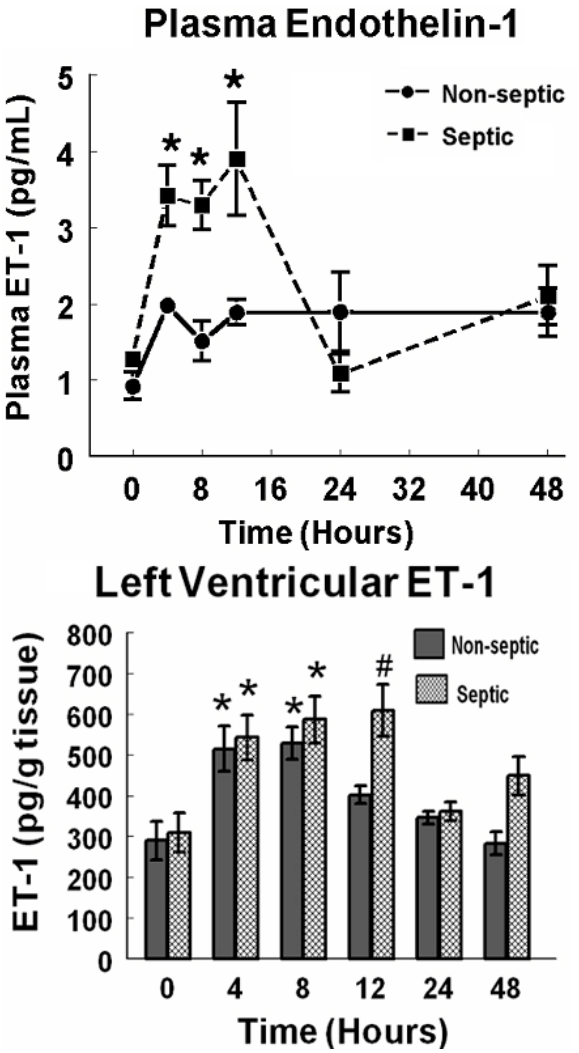

Figure 6. The concentration of ET-1 in plasma and left ventricular tissue at 0, 4, 8, 12, 24 and 48-h post-sepsis induction. * $P \leq 0.05$ as compared to baseline values and # $P \leq 0.05$ as compared to respective non-septic group.

the cytoplasm and regulates intracellular Ca^{2+} movements via receptors in the intracellular membrane system. DAG formed at the sarcolemma causes stimulation of protein kinase C (PKCs).

4.2.3. ET-1 and sepsis-induced myocardial Dysfunction

ET-1 is implicated in the regulation of regional blood flow and maintenance of vascular tone (84). ET-1 plays an important role in the pathogenesis of various diseases, including myocardial infarction (112), bronchial asthma (113), pulmonary hypertension (114), renal failure (115), and sepsis (13). Sharma *et al* (13), have demonstrated elevated plasma and myocardial ET-1 levels at 4, 8, 12-h post-sepsis induction that returned to baseline values 24-h later (Figure 6). In another study, we demonstrated that induction of sepsis produced early symptoms of CHF, as evident by elevated *tau* and increased myocardial work load, oxygen consumption, and LVEDP (57). In one study, we have used a physiological concentration of bigET-1 (which possesses less than one hundredth of the activity of ET-1 as a vasoconstrictor) to double bigET-1 levels in sham and sepsis, *in vivo* (44). This approach was used to determine if elevated ET-1(1-21) during early sepsis (13) initiates a protective or deteriorative physiological response. We found an elevated myocardial ET-1 concentration in the animals treated with bigET-1 without any significant elevation in plasma ET-1 concentrations in any of the groups studied. This suggests that increased levels of bigET-1 at an earlier time point may be triggering a higher expression of the ET-1 gene leading to mature ET-1 generation in the myocardium. BigET-1 treatment produced similar myocardial dysfunction (as evident by increased *tau* and LVEDP) both in sham and septic animals (57). This further supports our contention that an elevation of ET-1 in response to sepsis can be an important mediator affecting myocardial dysfunction seen later.

In ARVM, exogenous administration of the ET-1 precursor, bigET-1, elevated the concentration of ET-1 in supernatants and produced hypertrophy both in sham and sepsis groups. In addition, bigET-1 (100 nM) elevated ET-1 by 22 pg/g (~3 fold) in sham group vs. 16 pg/g (~6 fold) in sepsis group (116). Both endogenous ET-1 biosynthesis and exogenous availability of bigET-1 may account for the observed increase of ET-1 concentration in both sham and sepsis groups. Surprisingly, septic ARVM themselves have less basal ET-1. We speculate that this decreased ET-1 concentration in septic ARVM could be due to depressed intrinsic ECE-1 activity, reduced preproET-1 mRNA, or alterations in pretranscriptional regulation of the ET-1 gene. BigET-1 exerted a positive inotropic effect in sham ARVM and up to 3 hours in septic ARVM. However, septic ARVM did not exhibit any further alterations in PS, +dL/dt and –dL/dt following treatment of bigET-1 at 24 h post treatment. This non-responsive effect of the otherwise positive inotrope bigET-1 could be due to activation of various signaling molecules such as p38-MAPK or caspase-3 that have a negative inotropic influence on cardiomyocytes.

4.3. Signaling Cascade

4.3.1. Mitogen-activated protein kinases

Mammalian cells recognize and respond to extracellular stimuli via specific signaling cascades that activate mitogen activated-protein kinases (MAPKs), causing specific cellular responses. MAPKs are implicated in a gamut of cellular events such as mitosis, cell survival, apoptosis, cell differentiation, proliferation, etc. To date, five members of the MAPK family have been characterized in mammals: extracellular signal-regulated kinase1/2 (ERK1/2), p38-MAPK, c-Jun N-terminal kinase (JNK), big MAPK1 (ERK5), and ERKs 3 and 4 (117, 118). However, the most extensively studied MAPK members include ERK1/2, JNK, and p38-MAPK. A classification of the MAPK members has been developed by Philip Cohen, which provides a logical nomenclature for the various members of the MAPK family (Table 2). The MAPKs can be activated by a group of diverse stimuli. In general, the ERKs are predominantly activated by mitogenic and proliferative stimuli such as growth factors and hormones,

Table 2. MAPK nomenclature

Name	Cohen terminology	Phosphorylation motif	Alternate terminology	Substrates
ERK1 (44 kDa)	MAPK1	Thr – Glu - Tyr	p44-MAPK	MAPKAPK1, MNKs,MSKs, Elk1
ERK2 (42 kDa)	MAPK2	Thr – Glu – Tyr	p42-MAPK	MAPKAPK1, MNKs, MSKs, Elk1
JNK (~46 or ~54 kDa)	SAPK1a	Thr – Pro – Tyr	JNK2, SAPK-alpha	c-Jun, Jun D, ATF2, Elk1
JNK (~46 or ~54 kDa)	SAPK1b	Thr – Pro – Tyr	JNK3, SAPK-beta	c-Jun, Jun D, ATF2, Elk1
JNK (~46 or ~54 kDa)	SAPK1c	Thr – Pro – Tyr	JNK1, SAPK-gamma	c-Jun, Jun D, ATF2, Elk1
p38-MAPK (38 kDa)	SAPK2a	Thr – Gly - Tyr	p38-MAPK-alpha	MAPKAPK2/3, MSKs, ATF2, Elk1, MEF2C
p38-MAPKβ (38 kDa)	SAPK2b	Thr – Glu – Tyr		MAPKAPK2/3, MSKs, ATF2
p38-MAPKγ (38 kDa)	SAPK3	Thr – Gly – Tyr	ERK6	ATF-2
p38-MAPKδ (38 kDa)	SAPK4	Thr – Gly – Tyr		ATF-2
'Big' MAPK1	SAPK5	Thr – Glu - Tyr	ERK5, BMK 1	

MAPK, Mitogen-activated protein kinase; SAPK, Stress-activated protein kinase; JNK, c-Jun N-terminal kinase; Elk, ERK, Extracellular signal-regulated kinase; MAPKAPK, MAPK-activated protein kinase; ATF, Activating transcription factor; MNK, Mitogen-integrating kinase; MEK, MAPK kinase; MSK, Mitogen and stress activated protein kinases; MEF, MEK-enhancing factor; BMK, bigMAPK-1; Thr, Threonine; Glu, Glutamate; Tyr, Tyrosine; Pro, Proline; Gly, Glycine

while p38-MAPK and JNK regulate the cellular response to stress including ultraviolet light, osmotic stress, heat and inflammatory cytokines (119).

The MAPK family is highly conserved in all eukaryotes (117). Six diverse MAPKs have been identified in *Saccharomyces cerevisiae* (120). Thus, the possibility of the existence of additional mammalian MAPKs cannot be ruled out. Although each member of the MAPK superfamily has individual characteristics, several of the following common features are shared by all the members:

1. MAPK cascades are comprised of three conserved, sequentially acting protein kinases: MAPK, MAPK kinase (MAPKK or MKK) and MAPK kinase kinase (MAPKKK or MKKK) (Figure 7).

2. MAPKs are activated by the dual phosphorylation of Thr and Tyr residues in a 'T-Xaa-Y' motif (where Xaa= Glu for ERKs, Pro for JNK and Gly for p38-MAPK) by dual specific MAPKKs. The MAPKKs are themselves activated by MAPKKKs (Table 2).

3. MAPKKKs are serine/threonine kinases and are activated via phosphorylation of small GTP-binding proteins of the Ras/Rho family.

4. Once activated, MAPKS phosphorylate Ser-/Thr residues in target substrates within a (Ser-/Thr-) Pro consensus motif.

5. The MAPK cascade organization is also mediated by interaction with scaffolding proteins. Scaffold proteins bind and sequester selective MAPK components, which coordinate the activation and function of the MAPK components (121).

4.3.1.1. ERK1/2

The ERK1/2 cascade is the prototypical MAPK pathway. There are five members in this family: ERK1-5. ERK1 and ERK2, also known as p44-MAPK and p42-MAPK respectively, are the most widely studied MAPKs and most abundantly expressed isoforms (122-124). The ERK1/2 cascade is comprised of the MAPKKKs: A-raf, B-raf and Raf-1; MAPKKs: MEK1 and MEK2; and MAPKs: ERK1 and ERK2. ERK1/2 are potently activated by growth factors (fibroblast growth factor; FGF), phorbol esters (phorbol 12-myristate 13-acetate; PMA), ET-1 and alpha-adrenergic agonists (125). The knockouts for only ERK1 are well characterized. $ERK1^{-/-}$ mice appear normal and are viable with a modest defect in T-cell development (126). This suggests that the functions of ERK1 are conserved by ERK2. $MEK1^{-/-}$ mice die *in utero* exhibiting defective placental vascularization (127).

In cardiac myocytes, the ERK family of proteins is implicated in survival signaling in response to a horde of stimuli (124). It has also been postulated as a protective signaling mechanism against apoptosis (124). Mitogenic stimuli, such as growth factors, stimulate cell surface receptors, such as tyrosine kinases, that lead to the activation of small 21-kDa guanine nucleotide binding proteins (G proteins) Ras. The members of the Ras family include Ha-Ras, Ki-Ras (Ki-Ras4A and Ki-Ras4B), and N-Ras. Ras is localized to the plasma membrane due to lipid modifications such as farnesylation, carboxymethylation, and palmitoylation. Activation of Ras (Ras.GDP $\rightarrow$ Ras.GTP) involves stimulation of Sos, a guanine nucleotide exchange factor (GEF) (128). The activated Ras binds to c-Raf, causing its translocation to the membrane, where it gets activated. Activated Raf phosphorylates MEK1/2, which further activates ERK1/2. The precise mechanism of c-Raf activation is still controversial. Nevertheless, activation of c-Raf is crucial for the activation of ERK1/2. Activation of ERK1/2 is also mediated by GPCR agonists such as ET-1. The mechanism may involve DAG-sensitive PKC signaling. Peter Sugden (128) has suggested that PKCs activate Ras followed by c-Raf activation. However, additional receptors for DAG have been identified such as Ras.GRP (a GEF for Ras). Thus, Ras.GRPs may provide a PKC-independent mechanism for Ras activation in the ERK1/2 cascade.

It is now established that ERK1/2 regulates the growth response of cells. In cultured cardiac myocytes, ERK has been shown to be protective against apoptosis (129). Various studies suggest a role of ERK1/2 in cardiac myocyte hypertrophy (128). Evidence of compensated biventricular hypertrophy has been observed in transgenic mice, where ERK1/2 was activated by constitutively expressed MKK1. These mice showed improved myocardial contractility with resistance towards induction of apoptosis (130). Further evidence that demonstrated transient activation of ERK1/2 stimulates multiplication,

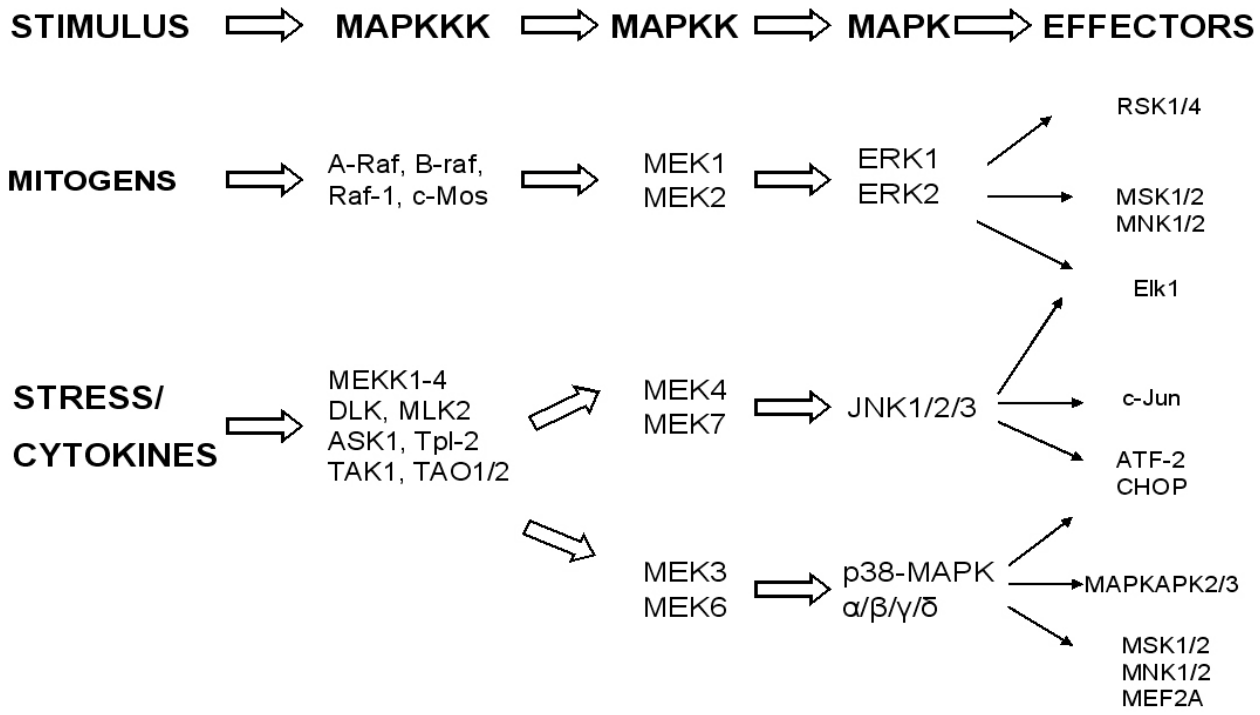

Figure 7. Various messenger systems involved in signal transduction pathways.

while sustained ERK1/2 activation can cause cell cycle arrest (131). However, the role of ERK 1/2 in the induction of apoptosis is still debatable.

The ERK1/2 cascade is generally associated with cell proliferation. In neuronal cells, sustained ERK1/2 activation by NGF induces differentiation. However, EGF produces transient ERK1/2 activation, ERK1/2 which contributes to the increased proliferative rate of tumor cells (132, 133). ERKs mediate responses in the central nervous system, such as regulation of learning and memory. MEK1 is also essential for the migration of vascular endothelial cells (127). Activation of ERK1/2 also modulates protein kinases (90-kDa ribosomal protein s6 kinases; p90RSKs) (134), transcription factors (Elk-1) (135), and other signaling proteins (phospholipase A2) (136).

In vivo, ERK1/2 activation has been shown to attenuate apoptosis subsequent to ischemia-reperfusion injury of the intact heart (137). In a polymicrobial septic rat model, we did not find any change in myocardial ERK1/2 expression at 24h post-sepsis induction, *in vivo* (23, 57). However, we observed that hyperglycemia produced an upregulation of ERK1/2 in septic rats (23). *In vitro* expression of activated ERK1/2 is significantly upregulated in septic ARVMs. However, treatment with bigET-1 did not further increase the expression of activated ERK1/2 in sepsis or in sham (116, 138). This suggests that bigET-1 does not modulate its effects through the ERK pathway in ARVM. Therefore, we concluded that during chronic sepsis, ERK1/2 may not play a significant role in sepsis-induced myocardial dysfunction.

4.3.1.2. p38-MAPK

The second member of the MAPK superfamily, p38-MAPKs, are activated by stress (arsenite, hypoxia/reoxygenation, ROS, hyperosmotic shock), UV, proinflammatory cytokines, and endotoxins (117, 139). The p38-MAPK cascade consists of MAPKKKs: MEKK1-4, MLK2 and –3, DLK, ASK1, Tpl2, and Tak1; MAPKKs: MEK3 (MKK3), and MEK5 (MKK6); MAPK: p38-MAPKs. The mammalian p38-MAPK is homologous to HOG1, the osmosensing MAPK of *S. cerevisiae* (140).

Several isoforms of p38-MAPK have been characterized: alpha-1/alpha-2 (141), beta-1/beta-2 (142, 143), gamma (144), and delta (145). The isoform p38-gamma is predominantly expressed in skeletal muscle, while p38-delta is widely expressed in various adult tissues and during development (146). p38-MAPK alpha and beta isoforms are more prevalent in the human heart than the other two isoforms. Human p38-alpha was originally purified and cloned as a polypeptide receptor for cytokine-suppressive anti-inflammatory drugs (CSAIDs) (141). Hence, p38-alpha is also termed as a CSAID-binding protein (CSBP). Among the four isoforms, only p38-MAPK alpha and beta are inhibited by CSAIDs, with the gamma and delta isoforms being non-responsive to these drugs (145). The knockout of only p38-MAPK alpha is available. Inactivation of p38-alpha causes extensive embryonic lethality (147). However, the severity and the cause of lethality vary with the genetic background in which p38-alpha deficiency is examined (148). $MKK3^{-/-}$ mice are viable without any obvious abnormalities (149). The macrophages of $MKK3^{-/-}$ mice exhibit reduced p38-MAPK activity by endotoxins (149).

Activation of p38-MAPK results by dual phosphorylation of Thr180 and Tyr182 by the upstream MAPKK, MKK6 and MKK3. MKK6 and MKK3 are in turn activated by several MAPKKKs described above in response to stress stimuli. MKK3 and –6 are highly specific for p38-MAPK activation and do not activate ERK1/2 and JNK. MKK6 activates all four isoforms of p38-MAPK, while MKK3 preferentially activates the p38-alpha and -beta isoforms. MKK4, on the other hand, activates JNK along with p38-MAPK, representing an upstream overlapping site for the p38-MAPK and JNK cascades.

Another mechanism has been suggested for the activation of p38-MAPK independent of the prototypic MAPKKs cascade (150). Interaction of p38-alpha with TAB1 (transforming growth factor-beta-activated protein kinase 1 (TAK1)-binding protein 1) produces intramolecular autophosphorylation and activation of p38-alpha. TAB1 is not a MKK and appears to be an adaptor or scaffolding protein with no known catalytic activity.

The biological functions of p38-MAPK include regulation of the immune and inflammatory responses, gene expression, interstitial remodeling, contractility, energy metabolism, and hormone synthesis (151, 152). Inhibition of p38-MAPK has been shown to downregulate the production of cytokines such as TNF-alpha, IL-1, etc. (153, 154). Mounting evidence suggests involvement of p38-MAPK in apoptosis, chemotaxis, granular exocytosis, cell differentiation, etc. (118, 151). Conflicting evidence also suggests an implication of p38-MAPK as a survival factor in contrast to being pro-apoptotic (139). The different isoforms of p38-MAPK also perform distinct biological functions. p38-MAPK alpha is pro-apoptotic (124), while p38-MAPK alpha induces a hypertrophic response. An upregulation of Bcl-2 protein expression in hearts of dominant-negative p38-alpha transgenic mice has been observed, suggesting a potential protective mechanism associated with p38 inhibition (155). Activation of p38-MAPK has also been shown to increase p53 protein levels, which in turn promotes apoptosis by inducing the expression and mitochondrial translocation of Bax (156, 157). Moreover, p38-MAPK is shown to translocate to the mitochondria of neuronal cells in response to nerve growth factor withdrawal, where it directly phosphorylates Bcl-2, inactivating its anti-apoptotic effects (158). p38-MAPK is known to localize to mitochondria in cardiac myocytes (159).

In the myocardium, activation of p38-MAPK has been observed in ischemia, ischemia/reperfusion (160), oxidative stress (139, 161), and heart failure (162, 163) in humans and animal models. MAPKs, particularly p38-MAPK, have been implicated in hypertrophy of cardiac myocytes (164, 165). However, in another study, dominant-negative mutant of p38-MAPK did not affect pressure overload-induced hypertrophy in cardiac myocytes but stimulated resistance to cardiac fibrosis (166). Inhibition of p38-MAPK activity *in vivo* attenuates cardiac remodelling and heart failure during myocardial infarction (167). Further, p38-MAPK has been shown to mediate a negative inotropic effect without altering intracellular calcium homeostasis in adult rat cardiac myocytes (ARVM) (168). In transgenic mice, p38-MAPK contributed to restrictive cardiomyopathy by induction of fetal gene expression, interstitial fibrosis, and loss of myocardial contractility (169). p38-MAPK also regulates cardiac cyclooxygenase-2 and prostaglandin biosynthesis (170, 171). However, the functions of p38-MAPK at the myocardium level are complex and are less clearly understood due to the presence of the various isoforms with diverse functions. Activation of p38-MAPK alpha in response to hypoxia has been shown to stabilize hypoxia-induced of erythropoietin (Epo) mRNA in human hepatoma cells (162) implicating the involvement of p38-MAPK in development. However, since the genetic knockouts of p38-MAPK alpha are lethal (147), the understanding of p38-MAPK phosphorylation in human pathophysiology is hindered.

Both *in vivo* and *in vitro* models of sepsis have shown an upregulation of phosphorylated p38-MAPK in ARVM and left ventricular tissue (57, 116). Treatment with bigET-1 caused an upregulation in p38 MAPK phosphorylation in sham and even further in sepsis. Septic ARVM, which showed depressed contractility, responded briefly to the positive inotropic effect of bigET-1. However, at 24 hours, at which point p38 MAPK phosphorylation is upregulated, septic ARVM did not show a response to bigET-1 (138). By inhibiting p38-MAPK with SB203580, bigET-1 exerted a positive inotropic effect in septic ARVM, but not to the same extent as seen in sham ARVM. This suggests that signaling mechanisms related to contractility are disrupted under septic conditions (138). Upon treatment of ARVM with FR901533, an ECE-1 inhibitor, and bigET-1, no significant change was seen in contractility despite a further upregulation of p38-MAPK phosphorylation (116). *In vivo*, bigET-1 has been shown to upregulate p38-MAPK phosphorylation in sham but not in sepsis. BigET-1 worsened the already existing myocardial dysfunction in sepsis as reflected by an increase in *tau* (57). Since p38-MAPK possesses a negative inotropic effect and activated for longer durations, its role in sepsis-induced myocardial dysfunction appears to be critical.

4.3.1.3. JNK

JNKs are another member of the MAPK superfamily. On a broader perspective, JNKS are members of the CMGC family of protein kinases of the human kinome (172). JNKs are also referred to by alternative nomenclature: 1) stress-activated protein kinases, because of their activation in response to stress; and 2) "JNK," in reference to their ability to phosphorylate the N-terminal of the transcription factor, c-Jun (117). JNKs are potently activated by stress, proinflammatory cytokines, UV, DNA-damaging agents, growth factors, etc. (117). Stress that activates JNK can be both cellular and mechanical. Cellular stresses include hyperosmotic shock, low concentrations of protein synthesis inhibitors (anisomycin), hypoxia/reoxygenation, and ROS (139). JNKs are not activated during global ischemia. Rather, their activation occurs during the reperfusion phase (160). Mechanical stresses that activate JNKs are passive stretch and electrical pacing. It is speculated that increasing the wall stress in intact hearts causes release of ET-1 and/or Ang II that

activates JNK (or p38-MAPK) in an autocrine/paracrine fashion (173).

Mammalian JNKs are encoded by three separate genes (JNK1, JNK2, and JNK3) localized on chromosomes 10q11.1-11.2, 5q35.3, and 4q21-g22.1, respectively (172). The three JNKs are further spliced to ten different proteins (174). Although all the JNKs are ubiquitously expressed, JNK3 is predominantly expressed in the brain. All the isoforms, however, differentially recognize and phosphorylate their transcription factor substrates (174). All three JNK loci have been knocked out. JNK1/2 $^{-/-}$ die at mid-gestation with defective neural-tube closure (175). Deletion of MKK4 causes abnormal liver development leading to mid-gestational lethality (176).

JNKs are activated by the dual phosphorylation on tyrosine and threonine residues within the conserved Thr-Pro-Tyr motif. The MAPKKs involved in the reaction are MKK4 (MEK4) and MKK7 (MEK7). These MAPKKs are themselves activated by various MAPKKKs, including MKKK1-4, MLK2 and –3, Tpl-2, DLK, TAO1 and –2, TAK1, and ASK1 and –2 (117). JNKs have been implicated in hypertrophy, apoptosis, cardiac remodeling, development, etc. (152). Wang *et al* (177) have demonstrated that activation of JNK via expression of activated MKK7 resulted in cardiac myocyte hypertrophy *in vitro*. Similarly, in cultured neonatal ventricular myocytes inhibition of JNK activity by expression of inactive MKK4 attenuated ET-1 induced hypertrophy in these myocytes (178). Dominant-negative JNK inhibited PE-induced ANF expression (179). Liang *et al* (180) have shown attenuation of hypertrophy by JNK in a model of pressure-overload-induced hypertrophy. Also, ASK-1$^{-/-}$ hearts show different effects on hypertrophy. Briefly, Ang II-induced cardiac hypertrophy was attenuated by reduced JNK activation (181). However, in the similar animals JNK reduced hypertrophy by pressure-overload (182). Thus, it appears that JNK may affect cardiac hypertrophy in a disparate manner depending on the nature of the hypertrophic stimuli.

JNK1 inhibition is reported to actually protect cardiac myocytes from ischemia-induced apoptosis, where as JNK2 inhibition had no effect (183). Activation of JNK has been shown to induce apoptosis in ARVM during oxidative stress (184). Similarly, increased apoptosis via JNK has also been observed in UV-irradiated fibroblasts (185). In contrast, a protective role of JNK1 signaling has been suggested during nitric oxide-induced cardiomyocyte apoptosis (186). JNKs have also been shown to attenuate apoptosis induced by nitric oxide (NO) in neonatal myocytes (172). However, the role of JNK signaling in regulation of apoptosis is still controversial.

4.4. Apoptosis cascade

Contrary to the earlier theory that cardiac myocytes perish due to necrosis, there have been numerous studies to establish the programmed death or apoptotic pathway in these vital cells. However, apoptosis in sepsis is still a mystery remaining to be deciphered. Septic myocytes can potentially apoptosize via activation of the death receptor mediated extrinsic pathway or the mitochondrial intrinsic pathway. The extrinsic machinery is a receptor-controlled phenomenon. It involves activation of cell surface death receptors by the tumor necrosis factor superfamily constituting extracellular ligands such as TNF-alpha and Fas ligand. The best characterized death receptors are Fas (CD95), TNF receptor -1 (TNFR1), and TRAIL (TNF-alpha related apoptosis inducing ligand) (187, 188). Since host response in sepsis stimulates proinflammatory cytokines such as TNF-alpha, induction of extrinsic pathway is inevitable. Interaction between the death receptor and ligand initiates the formation of a DISC (death-inducing signaling complex), along with procaspase-8. This results into an autophosphorylation reaction causing activation of downstream caspases such as caspase-3. Caspases possess DNase activity which ultimately results in DNA fragmentation (189). Anti-apoptotic X-linked inhibitor of apoptosis proteins (XIAP) can potentially prevent death receptor mediated apoptosis by binding to and inhibiting caspase-3 (190).

The intrinsic apoptotic cascade is a stress-activated pathway (191). Negative stress signals cause mitochondrial perturbation, resulting in the release of proteins that trigger the activation of caspases. Mitochondrial activity also culminates in disruption of electron transport and alteration of the cellular redox potential (192). Mitochondrial proteins having the greatest consequences are cytochrome C and second-mitochondria-derived activator of caspases (Smac). Once released cytochrome C, with the aid of ATP, induces the oligomerization of Apaf-1 to form the "apoptosome." This complex recruits the activation of initiator caspase-9, which amplifies the signal to stimulate effector caspases 3, -6, and 7 (193-195). Release of pro-apoptotic Bcl-2 proteins such as Bax promotes the permeabilization and disruption of the mitochondrial membrane (196). However, the cross-talk between the extrinsic and intrinsic pathway involves yet another Bcl-2 protein, Bid. Active caspase-8 can cleave this protein and thus cause its translocation from the cytosol to the outer mitochondrial membrane, stimulating the release of cytochrome C. Previously we demonstrated that sepsis upregulates p38-MAPK phosphorylation. This increase correlates with the activation of caspase-3, as seen in septic cardiomyocytes at 12 and 24 hr. We have also demonstrated that bigET-1 causes an increase in caspase-3 activity at 24 h in sham and septic myocytes (138). This increase in caspase-3 activity was not altered following treatment with a cell permeable caspase-3 inhibitor Ac-DEVD-CHO probably due to reversible inhibition of this drug. Also, treatment of the cells with captopril, a selective angiotensin converting enzyme inhibitor, did not alter caspase-3 activity (116).

These findings can also reflect the involvement of the extrinsic apoptosis pathway in septic myocytes. Our findings also corroborate that p38-MAPK exerts pro-apoptotic effects, as suggested by Gonzalez *et al* (197). ASK-1 is a member of the MAPK family that activates JNK & p38-MAPK and executes apoptosis by mitochondria dependent caspase-3 activation, which involves the phosphorylation of Bcl_2 along with the release

of cytochrome C (198). ATF-2, a downstream effector in the MAPK cascade that is a member of the ATF/cAMP response element binding protein family, also plays an important role in cellular stress response (199). Activation of p38-MAPK mediated upregulation of Bax, Bcl_2, and activation of caspase-3 has been reported as one of the main pathways for apoptosis and hypertrophy in ARVM (200). Activation of the innate immune system following sepsis initiates the release of pro-inflammatory cytokines, which act on TLRs (toll like receptors). NF-kB is the central downstream target of the TLR-mediated pathway. NF-kB related genes have been implicated in the pathogenesis of cardiac dysfunction in sepsis. Several MAPKs including JNK and p38-MAPK also cause phosphorylation of NF-kB.

4.4.1. Role of calcineurin

Calcineurin (PP2B) is a serine/threonine protein phosphatase that responds to elevated intracellular calcium (201-203). On activation, calcineurin dephosphorylates members of the NFAT transcription factors in the cytoplasm. Dephosphorylated NFAT translocates to the nucleus, where it has been shown to play an important role in regulating the hypertrophic growth response (204). Calcineurin's role in apoptosis is controversial as in some cell types it agonizes, while in others it antagonizes apoptosis as reported (124). Calcineurin is reported to function in a way that its transient activation antagonizes myocytes apoptosis. However, its prolonged activation induces cardiac hypertrophy and deleterious effect on the heart (124). Calcineurin has been proposed to play a role in the activation of iNOS in LPS-stimulated mouse peritoneal macrophages, but its role in chronic sepsis-induced myocardial dysfunction *in vivo* is not known (205).

4.4.2. Protein kinase C (PKC) signaling

PKC is a family of 11 serine/threonine kinases, most of which have been identified in the heart (206-208). These 11 enzymes are subdivided into three groups: classical PKCs are those which are activated in presence of Ca2+ and DAG; novel PKCs that are activated by only DAG; and atypical PKCs which are insensitive to Ca2+ or DAG but are activated by certain lipids (206). Classically, G-protein coupled receptors (GPCRs) activate PKC, which in turn hydrolyzes phospholipids and elevates intracellular DAG and calcium (206). However, PKC activation can also occur through growth factor receptors, nitric oxide and reactive oxygen species in the myocardium (209-211). PKCδ and PKCε have distinct effects on cardiac myocyte survival and death (124). In cardiac myocytes PKCδ has been found to selectively activate the JNK and p38-MAPK pathways, whereas PKCε activates the ERK pathway (212). Cardioprotection mediated through PKCε has been shown to be associated with an anti-apoptotic kinase Akt (213). Translocation of PKCδ to mitochondria is essential for initiation of mitochondrial death pathway (214-217). PKCε, on the other hand, can prevent mitochondria-mediated cell death (218, 219). In our earlier study, we could not observe any alteration in the expression of myocardial PKCε at 24-h post-LPS administration, *in vivo* (220). It appears that the activation of PKC-ε can play a role during very early activation of sepsis-induced signaling molecules or mediators. However, its precise role needs to be elucidated further.

Other regulatory kinases like protein kinase A (PKA) and calcium/calmodulin kinase (CaMK) have been shown to play a role in myocardial apoptosis (124). However, their specific roles during sepsis-induced myocardial dysfunction are far from clear.

5. FUTURE PROSPECTIVES

The data from our group demonstrate that ET-1 precursor produces decompensatory hypertrophy in ventricular myocytes and contractile dysfunction (hyporesponsiveness to bigET-1). The role of ET-1 on mitochondrial oxidation cascade and extrinsic apotosis during sepsis is not yet clear. We suspect that poor understanding of myocardial signaling during early sepsis could be one of the main reasons for limited success of pharmacotherapeutic options for sepsis. It appears that signaling cascade activation, apoptosis, and contractility alterations during sepsis needs to be thoroughly elucidated so as to limit the myocardial damage caused by sepsis, septic shock, and related cardiovascular disorders.

6. ACKNOWLEDGMENTS

The research work in Dr. Sharma's laboratory is supported by NIH, HL066016, American Heart Association Greater Midwest Beginning Grant-In-Aid (AHA#0160444Z). SB received a Predoctoral fellowship from American Heart Association Greater Midwest affiliate (AHA#0510075Z) and AG received a Presidential fellowship from North Dakota State University.

7. REFERENCES

1. Bone, R. C.: Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. *Crit Care Med* 24, 163-72 (1996)
2. Friedman, G., E. Silva & J. L. Vincent: Has the mortality of septic shock changed with time. *Crit Care Med* 26, 2078-86 (1998)
3. Balk, R. A.: Severe sepsis and septic shock. Definitions, epidemiology, and clinical manifestations. *Crit Care Clin* 16, 179-92 (2000)
4. Wenzel, R. P. & M. B. Edmond: Managing antibiotic resistance. *N Engl J Med* 343, 1961-3 (2000)
5. Hoyert, D. L., H. C. Kung & B. L. Smith: Deaths: Preliminary data for 2003. National Center for Health Statistics, 1-48 (2005).
6. Deitch, E. A.: Animal models of sepsis and shock: a review and lessons learned. *Shock* 9, 1-11 (1998)
7. Wichterman, K. A., A. E. Baue & I. H. Chaudry: Sepsis and septic shock--a review of laboratory models and a proposal. *J Surg Res* 29, 189-201 (1980)
8. Fink, M. P. & S. O. Heard: Laboratory models of sepsis and septic shock. *J Surg Res* 49, 186-96 (1990)
9. Cross, A. S., S. M. Opal, J. C. Sadoff & P. Gemski: Choice of bacteria in animal models of sepsis. *Infect Immun* 61, 2741-7 (1993)

10. Fink, M. P., T. J. MacVittie & L. C. Casey: Inhibition of prostaglandin synthesis restores normal hemodynamics in canine hyperdynamic sepsis. *Ann Surg* 200, 619-26 (1984)
11. Kazarian, K. K., P. W. Perdue, W. Lynch, A. Dziki, J. Nevola, C. H. Lee, I. Hayward, T. Williams & W. R. Law: Porcine peritoneal sepsis: modeling for clinical relevance. *Shock* 1, 201-12 (1994)
12. Sharma, A. C., H. B. Bosmann, S. J. Motew, K. H. Hales, D. B. Hales & J. L. Ferguson: Steroid hormone alterations following induction of chronic intraperitoneal sepsis in male rats. *Shock* 6, 150-4 (1996)
13. Sharma, A. C., S. J. Motew, S. Farias, K. J. Alden, H. B. Bosmann, W. R. Law & J. L. Ferguson: Sepsis alters myocardial and plasma concentrations of endothelin and nitric oxide in rats. *J Mol Cell Cardiol* 29, 1469-77 (1997)
14. Sam, A. D., 2nd, A. C. Sharma, W. R. Law & J. L. Ferguson: Splanchnic vascular control during sepsis and endotoxemia. *Front Biosci* 2, e72-92 (1997)
15. Alden, K. J., S. J. Motew, A. C. Sharma & J. L. Ferguson: Effect of aminoguanidine on plasma nitric oxide by-products and blood flow during chronic peritoneal sepsis. *Shock* 9, 289-95 (1998)
16. Sharma, A. C., A. D. Sam, 2nd, L. Y. Lee, D. B. Hales, W. R. Law, J. L. Ferguson & H. B. Bosmann: Effect of NG-nitro-L-arginine methyl ester on testicular blood flow and serum steroid hormones during sepsis. *Shock* 9, 416-21 (1998)
17. Motew, S. J., A. D. Sam, M. G. Mourelatos, A. C. Sharma, K. J. Alden, J. L. Ferguson & W. R. Law: Adenosine receptor antagonism affects regional resting vascular resistance during rat peritoneal sepsis. *J Surg Res* 80, 326-32 (1998)
18. Sam, A. D., 2nd, A. C. Sharma, L. Y. Lee, D. B. Hales, W. R. Law, J. L. Ferguson & H. B. Bosmann: Sepsis produces depression of testosterone and steroidogenic acute regulatory (StAR) protein. *Shock* 11, 298-301 (1999)
19. Sam, A. D., 2nd, A. C. Sharma, A. N. Rice, J. L. Ferguson & W. R. Law: Interdependence of adenosine and nitric oxide in hepato-splanchnic circulation during sepsis. *J Surg Res* 94, 61-7 (2000)
20. Sam, A. D., 2nd, A. C. Sharma, A. N. Rice, J. L. Ferguson & W. R. Law: Adenosine and nitric oxide regulate regional vascular resistance via interdependent and independent mechanisms during sepsis. *Crit Care Med* 28, 1931-9 (2000)
21. Omachi, A., A. C. Sharma, K. J. Alden, A. D. Sam & J. L. Ferguson: Induction of peritoneal sepsis increases the susceptibility of isolated hearts to a calcium paradox-mediated injury. *Shock* 17, 193-8 (2002)
22. Ren, J., B. H. Ren & A. C. Sharma: Sepsis-induced depressed contractile function of isolated ventricular myocytes is due to altered calcium transient properties. *Shock* 18, 285-8 (2002)
23. Gupta, A., S. Brahmbhatt & A. C. Sharma: Left ventricular mitogen activated protein kinase signaling following polymicrobial sepsis during streptozotocin-induced hyperglycemia. *Biochim Biophys Acta* 1690, 42-53 (2004)
24. Law, W. R., V. E. Valli & B. A. Conlon: Therapeutic potential for transient inhibition of adenosine deaminase in systemic inflammatory response syndrome. *Crit Care Med* 31, 1475-81 (2003)
25. Adanin, S., I. V. Yalovetskiy, B. A. Nardulli, A. D. Sam, 2nd, Z. S. Jonjev & W. R. Law: Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis. *Am J Physiol Regul Integr Comp Physiol* 282, R1324-32 (2002)
26. Schrier, R. W. & W. Wang: Acute renal failure and sepsis. *N Engl J Med* 351, 159-69 (2004)
27. MacLean, L. D., W. G. Mulligan, A. P. McLean & J. H. Duff: Patterns of septic shock in man--a detailed study of 56 patients. *Ann Surg* 166, 543-62 (1967)
28. Clowes, G. H., Jr., M. Vucinic & M. G. Weidner: Circulatory and metabolic alterations associated with survival or death in peritonitis: clinical analysis of 25 cases. *Ann Surg* 163, 866-85 (1966)
29. Nishijima, H., M. H. Weil, H. Shubin & J. Cavanilles: Hemodynamic and metabolic studies on shock associated with gram negative bacteremia. *Medicine (Baltimore)* 52, 287-94 (1973)
30. Kumar, A., C. Haery & J. E. Parrillo: Myocardial dysfunction in septic shock. *Crit Care Clin* 16, 251-87 (2000)
31. Court, O., A. Kumar & J. E. Parrillo: Clinical review: Myocardial depression in sepsis and septic shock. *Crit Care* 6, 500-8 (2002)
32. Ceneviva, G., J. A. Paschall, F. Maffei & J. A. Carcillo: Hemodynamic support in fluid-refractory pediatric septic shock. *Pediatrics* 102, e19 (1998)
33. Weisel, R. D., L. Vito, R. C. Dennis, C. R. Valeri & H. B. Hechtman: Myocardial depression during sepsis. *Am J Surg* 133, 512-21 (1977)
34. Calvin, J. E., A. A. Driedger & W. J. Sibbald: An assessment of myocardial function in human sepsis utilizing ECG gated cardiac scintigraphy. *Chest* 80, 579-86 (1981)
35. Parker, M. M., J. H. Shelhamer, S. L. Bacharach, M. V. Green, C. Natanson, T. M. Frederick, B. A. Damske & J. E. Parrillo: Profound but reversible myocardial depression in patients with septic shock. *Ann Intern Med* 100, 483-90 (1984)
36. Jafri, S. M., S. Lavine, B. E. Field, M. T. Bahorozian & R. W. Carlson: Left ventricular diastolic function in sepsis. *Crit Care Med* 18, 709-14 (1990)
37. Munt, B., J. Jue, K. Gin, J. Fenwick & M. Tweeddale: Diastolic filling in human severe sepsis: an echocardiographic study. *Crit Care Med* 26, 1829-33 (1998)
38. Poelaert, J., C. Declerck, D. Vogelaers, F. Colardyn & C. A. Visser: Left ventricular systolic and diastolic function in septic shock. *Intensive Care Med* 23, 553-60 (1997)
39. Parker, M. M., K. E. McCarthy, F. P. Ognibene & J. E. Parrillo: Right ventricular dysfunction and dilatation, similar to left ventricular changes, characterize the cardiac depression of septic shock in humans. *Chest* 97, 126-31 (1990)
40. Parker, M. M., J. H. Shelhamer, C. Natanson, D. W. Alling & J. E. Parrillo: Serial cardiovascular variables in survivors and nonsurvivors of human septic shock: heart rate as an early predictor of prognosis. *Crit Care Med* 15, 923-9 (1987)
41. Krausz, M. M., A. Perel, D. Eimerl & S. Cotev: Cardiopulmonary effects of volume loading in patients in septic shock. *Ann Surg* 185, 429-34 (1977)
42. Rhodes, A., F. J. Lamb, I. Malagon, P. J. Newman, R. M. Grounds & E. D. Bennett: A prospective study of the use of a dobutamine stress test to identify outcome in patients with sepsis, severe sepsis, or septic shock. *Crit Care Med* 27, 2361-6 (1999)

43. McDonough, K. H., T. Smith, K. Patel & M. Quinn: Myocardial dysfunction in the septic rat heart: role of nitric oxide. *Shock* 10, 371-6 (1998)
44. Tschaikowsky, K., S. Sagner, N. Lehnert, M. Kaul & J. Ritter: Endothelin in septic patients: effects on cardiovascular and renal function and its relationship to proinflammatory cytokines. *Crit Care Med* 28, 1854-60 (2000)
45. Krishnagopalan, S., A. Kumar & J. E. Parrillo: Myocardial dysfunction in the patient with sepsis. *Curr Opin Crit Care* 8, 376-88 (2002)
46. Landgarten, M. J., A. Kumar & J. E. Parrillo: Cardiovascular Dysfunction in Sepsis and Septic Shock. *Curr Treat Options Cardiovasc Med* 2, 451-459 (2000)
47. Kuebler, J. F., D. Jarrar, B. Toth, K. I. Bland, L. Rue, 3rd, P. Wang & I. H. Chaudry: Estradiol administration improves splanchnic perfusion following trauma-hemorrhage and sepsis. *Arch Surg* 137, 74-9 (2002)
48. Opie, L. H.: The heart, physiology from cell to circulation. Raven Publishers, Philadelphia (1998)
49. Stahl, T. J., P. B. Alden, W. S. Ring, R. C. Madoff & F. B. Cerra: Sepsis-induced diastolic dysfunction in chronic canine peritonitis. *Am J Physiol* 258, H625-33 (1990)
50. Piper, R. D., F. Y. Li, M. L. Myers & W. J. Sibbald: Structure-function relationships in the septic rat heart. *Am J Respir Crit Care Med* 156, 1473-82 (1997)
51. Field, B. E., E. C. Rackow, M. E. Astiz & M. H. Weil: Early systolic and diastolic dysfunction during sepsis in rats. *J Crit Care* 4, 3-8 (1989)
52. Guntheroth, W. G., J. P. Jacky, I. Kawabori, J. G. Stevenson & A. H. Moreno: Left ventricular performance in endotoxin shock in dogs. *Am J Physiol* 242, H172-6 (1982)
53. Werner, H. A., M. J. Herbertson & K. R. Walley: Amrinone increases ventricular contractility and diastolic compliance in endotoxemia. *Am J Respir Crit Care Med* 152, 496-503 (1995)
54. Zhong, J., L. J. Rubin, J. L. Parker & H. R. Adams: Cardiodynamic response to Escherichia coli endotoxemia: effects of fluid resuscitation. *Shock* 2, 203-9 (1994)
55. Adams, H. R., C. R. Baxter & J. L. Parker: Reduction of intrinsic contractile reserves of the left ventricle by Escherichia coli endotoxin shock in guinea-pigs. *J Mol Cell Cardiol* 17, 575-85 (1985)
56. Raymond, R. M.: When does the heart fail during shock? *Circ Shock* 30, 27-41 (1990)
57. Brahmbhatt, S., A. Gupta & A. C. Sharma: Bigendothelin-1 (1-21) fragment during early sepsis modulates tau, p38-MAPK phosphorylation and nitric oxide synthase activation. *Mol Cell Biochem* 271, 225-37 (2005)
58. Serizawa, T., B. A. Carabello & W. Grossman: Effect of pacing-induced ischemia on left ventricular diastolic pressure-volume relations in dogs with coronary stenoses. *Circ Res* 46, 430-9 (1980)
59. Miyamoto, M. I., G. A. Rose, N. J. Weissman, J. L. Guerrero, M. J. Semigran & M. H. Picard: Abnormal global left ventricular relaxation occurs early during the development of pharmacologically induced ischemia. *J Am Soc Echocardiogr* 12, 113-20 (1999)
60. Fifer, M. A., P. D. Bourdillon & B. H. Lorell: Altered left ventricular diastolic properties during pacing-induced angina in patients with aortic stenosis. *Circulation* 74, 675-83 (1986)
61. Farias, S., F. M. Powers & W. R. Law: End-diastolic pressure-volume relationship in sepsis: relative contributions of compliance and equilibrium chamber volume differ. *J Surg Res* 82, 172-9 (1999)
62. Garner, L. B., M. S. Willis, D. L. Carlson, J. M. DiMaio, M. D. White, D. J. White, G. A. t. Adams, J. W. Horton & B. P. Giroir: Macrophage migration inhibitory factor is a cardiac-derived myocardial depressant factor. *Am J Physiol Heart Circ Physiol* 285, H2500-9 (2003)
63. Matsukawa, A., C. M. Hogaboam, N. W. Lukacs, P. M. Lincoln, H. L. Evanoff & S. L. Kunkel: Pivotal role of the CC chemokine, macrophage-derived chemokine, in the innate immune response. *J Immunol* 164, 5362-8 (2000)
64. Sharma, G. V., P. A. Woods, C. T. Lambrew, C. M. Berg, D. A. Pietro, T. P. Rocco, F. W. Welt, P. Sacchetti & K. M. McIntyre: Evaluation of a noninvasive system for determining left ventricular filling pressure. *Arch Intern Med* 162, 2084-8 (2002)
65. Lambermont, B., A. Ghuysen, P. Kolh, V. Tchana-Sato, P. Segers, P. Gerard, P. Morimont, D. Magis, J. M. Dogne, B. Masereel & V. D'Orio: Effects of endotoxic shock on right ventricular systolic function and mechanical efficiency. *Cardiovasc Res* 59, 412-8 (2003)
66. Strauer, B. E.: Left ventricular dynamics, energetics and coronary hemodynamics in hypertrophic heart disease. *Eur Heart J* 4 Suppl A, 137-42 (1983)
67. Francis, G. S. & J. N. Cohn: Congestive heart failure: Pathophysiology and therapy. Raven Press, New York (1990)
68. Meldrum, D. R.: Tumor necrosis factor in the heart. *Am J Physiol* 274, R577-95 (1998)
69. Lo, C. J., I. Garcia, H. G. Cryer & R. V. Maier: Calcium and calmodulin regulate lipopolysaccharide-induced alveolar macrophage production of tumor necrosis factor and procoagulant activity. *Arch Surg* 131, 44-50 (1996)
70. Horton, J. W., D. Maass, J. White & B. Sanders: Nitric oxide modulation of TNF-alpha-induced cardiac contractile dysfunction is concentration dependent. *Am J Physiol Heart Circ Physiol* 278, H1955-65 (2000)
71. Bryant, D., L. Becker, J. Richardson, J. Shelton, F. Franco, R. Peshock, M. Thompson & B. Giroir: Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. *Circulation* 97, 1375-81 (1998)
72. Cohen, J.: The immunopathogenesis of sepsis. *Nature* 420, 885-91 (2002)
73. Beutler, B. A., I. W. Milsark & A. Cerami: Cachectin/tumor necrosis factor: production, distribution, and metabolic fate *in vivo*. *J Immunol* 135, 3972-7 (1985)
74. Michie, H. R., K. R. Manogue, D. R. Spriggs, A. Revhaug, S. O'Dwyer, C. A. Dinarello, A. Cerami, S. M. Wolff & D. W. Wilmore: Detection of circulating tumor necrosis factor after endotoxin administration. *N Engl J Med* 318, 1481-6 (1988)
75. Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry & A. Cerami: Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 330, 662-4 (1987)
76. Borrelli, E., P. Roux-Lombard, G. E. Grau, E. Girardin, B. Ricou, J. Dayer & P. M. Suter: Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins

can predict the development of multiple organ failure in patients at risk. *Crit Care Med* 24, 392-7 (1996)
77. Fisher, C. J., Jr., J. M. Agosti, S. M. Opal, S. F. Lowry, R. A. Balk, J. C. Sadoff, E. Abraham, R. M. Schein & E. Benjamin: Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N Engl J Med* 334, 1697-702 (1996)
78. Abraham, E., M. P. Glauser, T. Butler, J. Garbino, D. Gelmont, P. F. Laterre, K. Kudsk, H. A. Bruining, C. Otto, E. Tobin, C. Zwingelstein, W. Lesslauer & A. Leighton: p55 Tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. A randomized controlled multicenter trial. Ro 45-2081 Study Group. *Jama* 277, 1531-8 (1997)
79. Abraham, E., A. Anzueto, G. Gutierrez, S. Tessler, G. San Pedro, R. Wunderink, A. Dal Nogare, S. Nasraway, S. Berman, R. Cooney, H. Levy, R. Baughman, M. Rumbak, R. B. Light, L. Poole, R. Allred, J. Constant, J. Pennington & S. Porter: Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. *Lancet* 351, 929-33 (1998)
80. Francis, S. E., H. Holden, C. M. Holt & G. W. Duff: Interleukin-1 in myocardium and coronary arteries of patients with dilated cardiomyopathy. *J Mol Cell Cardiol* 30, 215-23 (1998)
81. Fisher, C. J., Jr., J. F. Dhainaut, S. M. Opal, J. P. Pribble, R. A. Balk, G. J. Slotman, T. J. Iberti, E. C. Rackow, M. J. Shapiro, R. L. Greenman & *et al.*: Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *Jama* 271, 1836-43 (1994)
82. Opal, S. M., C. J. Fisher, Jr., J. F. Dhainaut, J. L. Vincent, R. Brase, S. F. Lowry, J. C. Sadoff, G. J. Slotman, H. Levy, R. A. Balk, M. P. Shelly, J. P. Pribble, J. F. LaBrecque, J. Lookabaugh, H. Donovan, H. Dubin, R. Baughman, J. Norman, E. DeMaria, K. Matzel, E. Abraham & M. Seneff: Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. *Crit Care Med* 25, 1115-24 (1997)
83. Damas, P., D. Ledoux, M. Nys, Y. Vrindts, D. De Groote, P. Franchimont & M. Lamy: Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. *Ann Surg* 215, 356-62 (1992)
84. Sharma, A. C. & A. Gulati: Role of endothelin in regional vascular system. Harwood Academic Publishers gmbh, Amsterdam (1995)
85. Battistini, B., M. A. Forget & D. Laight: Potential roles for endothelins in systemic inflammatory response syndrome with a particular relationship to cytokines. *Shock* 5, 167-83 (1996)
86. Hemsen, A.: Biochemical and functional characterization of endothelin peptides with special reference to vascular effects. *Acta Physiol Scand Suppl* 602, 1-61 (1991)
87. Kaddoura, S., N. P. Curzen, T. W. Evans, J. D. Firth & P. A. Poole-Wilson: Tissue expression of endothelin-1 mRNA in endotoxaemia. *Biochem Biophys Res Commun* 218, 641-7 (1996)
88. Weitzberg, E., G. Ahlborg & J. M. Lundberg: Long-lasting vasoconstriction and efficient regional extraction of endothelin-1 in human splanchnic and renal tissues. *Biochem Biophys Res Commun* 180, 1298-303 (1991)
89. Weitzberg, E., G. Ahlborg & J. M. Lundberg: Differences in vascular effects and removal of endothelin-1 in human lung, brain, and skeletal muscle. *Clin Physiol* 13, 653-62 (1993)
90. Pittet, J. F., D. R. Morel, A. Hemsen, K. Gunning, J. S. Lacroix, P. M. Suter & J. M. Lundberg: Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. *Ann Surg* 213, 261-4 (1991)
91. Oldner, A., M. Wanecek, E. Weitzberg, M. Rundgren, K. Alving, J. Ullman & A. Rudehill: Angiotensin II receptor antagonism increases gut oxygen delivery but fails to improve intestinal mucosal acidosis in porcine endotoxin shock. *Shock* 11, 127-35 (1999)
92. Hickey, K. A., G. Rubanyi, R. J. Paul & R. F. Highsmith: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am J Physiol* 248, C550-6 (1985)
93. Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto & T. Masaki: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332, 411-5 (1988)
94. Rubanyi, G. M. & M. A. Polokoff: Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol Rev* 46, 325-415 (1994)
95. Arinami, T., M. Ishikawa, A. Inoue, M. Yanagisawa, T. Masaki, M. C. Yoshida & H. Hamaguchi: Chromosomal assignments of the human endothelin family genes: the endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. *Am J Hum Genet* 48, 990-6 (1991)
96. Bloch, K. D., R. L. Eddy, T. B. Shows & T. Quertermous: cDNA cloning and chromosomal assignment of the gene encoding endothelin 3. *J Biol Chem* 264, 18156-61 (1989)
97. Levin, E. R.: Endothelins. *N Engl J Med* 333, 356-63 (1995)
98. Shinmi, O., S. Kimura, T. Sawamura, Y. Sugita, T. Yoshizawa, Y. Uchiyama, M. Yanagisawa, K. Goto, T. Masaki & I. Kanazawa: Endothelin-3 is a novel neuropeptide: isolation and sequence determination of endothelin-1 and endothelin-3 in porcine brain. *Biochem Biophys Res Commun* 164, 587-93 (1989)
99. Inoue, A., M. Yanagisawa, Y. Takuwa, Y. Mitsui, M. Kobayashi & T. Masaki: The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J Biol Chem* 264, 14954-9 (1989)
100. Kido, T., T. Sawamura, H. Hoshikawa, P. D'Orleans-Juste, J. B. Denault, R. Leduc, J. Kimura & T. Masaki: Processing of proendothelin-1 at the C-terminus of big endothelin-1 is essential for proteolysis by endothelin-converting enzyme-1 *in vivo*. *Eur J Biochem* 244, 520-6 (1997)
101. Kimura, S., Y. Kasuya, T. Sawamura, O. Shinimi, Y. Sugita, M. Yanagisawa, K. Goto & T. Masaki: Conversion of big endothelin-1 to 21-residue endothelin-1 is essential for expression of full vasoconstrictor activity: structure-activity relationships of big endothelin-1. *J Cardiovasc Pharmacol* 13 Suppl 5, S5-7; discussion S18 (1989)

102. Arai, H., S. Hori, I. Aramori, H. Ohkubo & S. Nakanishi: Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* 348, 730-2 (1990)
103. Sakurai, T., M. Yanagisawa, Y. Takuwa, H. Miyazaki, S. Kimura, K. Goto & T. Masaki: Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* 348, 732-5 (1990)
104. Karne, S., C. K. Jayawickreme & M. R. Lerner: Cloning and characterization of an endothelin-3 specific receptor (ETC receptor) from Xenopus laevis dermal melanophores. *J Biol Chem* 268, 19126-33 (1993)
105. Hosoda, K., K. Nakao, A. Hiroshi, S. Suga, Y. Ogawa, M. Mukoyama, G. Shirakami, Y. Saito, S. Nakanishi & H. Imura: Cloning and expression of human endothelin-1 receptor cDNA. *FEBS Lett* 287, 23-6 (1991)
106. Hilal-Dandan, R., M. T. Ramirez, S. Villegas, A. Gonzalez, Y. Endo-Mochizuki, J. H. Brown & L. L. Brunton: Endothelin ETA receptor regulates signaling and ANF gene expression via multiple G protein-linked pathways. *Am J Physiol* 272, H130-7 (1997)
107. Boivin, B., D. Chevalier, L. R. Villeneuve, E. Rousseau & B. G. Allen: Functional endothelin receptors are present on nuclei in cardiac ventricular myocytes. *J Biol Chem* 278, 29153-63 (2003)
108. Pierce, K. L., R. T. Premont & R. J. Lefkowitz: Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* 3, 639-50 (2002)
109. Fukami, K.: Structure, regulation, and function of phospholipase C isozymes. *J Biochem (Tokyo)* 131, 293-9 (2002)
110. Clerk, A. & P. H. Sugden: Regulation of phospholipases C and D in rat ventricular myocytes: stimulation by endothelin-1, bradykinin and phenylephrine. *J Mol Cell Cardiol* 29, 1593-604 (1997)
111. Ross, E. M. & T. M. Wilkie: GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem* 69, 795-827 (2000)
112. Taylor, A. J., A. Bobik, M. Richards, D. Kaye, G. Raines, P. Gould & G. Jennings: Myocardial endothelin-1 release and indices of inflammation during angioplasty for acute myocardial infarction and stable coronary artery disease. *Am Heart J* 148, e10 (2004)
113. Nikolaou, E., G. Trakada, E. Prodromakis, G. Efremidis, A. Pouli, A. Koniavitou & K. Spiropoulos: Evaluation of arterial endothelin-1 levels, before and during a sleep study, in patients with bronchial asthma and chronic obstructive pulmonary disease. *Respiration* 70, 606-10 (2003)
114. Channick, R. N., O. Sitbon, R. J. Barst, A. Manes & L. J. Rubin: Endothelin receptor antagonists in pulmonary arterial hypertension. *J Am Coll Cardiol* 43, 62S-67S (2004)
115. Goddard, J., N. R. Johnston, M. F. Hand, A. D. Cumming, T. J. Rabelink, A. J. Rankin & D. J. Webb: Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: a comparison of selective and combined endothelin receptor blockade. *Circulation* 109, 1186-93 (2004)
116. Gupta, A., N. S. Aberle, 2nd, J. Ren & A. C. Sharma: Endothelin-converting enzyme-1-mediated signaling in adult rat ventricular myocyte contractility and apoptosis during sepsis. *J Mol Cell Cardiol* 38, 527-37 (2005)
117. Kyriakis, J. M. & J. Avruch: Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 81, 807-69 (2001)
118. Roux, P. P. & J. Blenis: ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 68, 320-44 (2004)
119. Pearson, G., F. Robinson, T. Beers Gibson, B. E. Xu, M. Karandikar, K. Berman & M. H. Cobb: Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 22, 153-83 (2001)
120. Madhani, H. D. & G. R. Fink: The riddle of MAP kinase signaling specificity. *Trends Genet* 14, 151-5 (1998)
121. Pawson, T. & J. D. Scott: Signaling through scaffold, anchoring, and adaptor proteins. *Science* 278, 2075-80 (1997)
122. Garrington, T. P. & G. L. Johnson: Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol* 11, 211-8 (1999)
123. Widmann, C., S. Gibson, M. B. Jarpe & G. L. Johnson: Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 79, 143-80 (1999)
124. Baines, C. P. & J. D. Molkentin: STRESS signaling pathways that modulate cardiac myocyte apoptosis. *J Mol Cell Cardiol* 38, 47-62 (2005)
125. Clerk, A., M. A. Bogoyevitch, M. B. Anderson & P. H. Sugden: Differential activation of protein kinase C isoforms by endothelin-1 and phenylephrine and subsequent stimulation of p42 and p44 mitogen-activated protein kinases in ventricular myocytes cultured from neonatal rat hearts. *J Biol Chem* 269, 32848-57 (1994)
126. Pages, G., S. Guerin, D. Grall, F. Bonino, A. Smith, F. Anjuere, P. Auberger & J. Pouyssegur: Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. *Science* 286, 1374-7 (1999)
127. Giroux, S., M. Tremblay, D. Bernard, J. F. Cardin-Girard, S. Aubry, L. Larouche, S. Rousseau, J. Huot, J. Landry, L. Jeannotte & J. Charron: Embryonic death of Mek1-deficient mice reveals a role for this kinase in angiogenesis in the labyrinthine region of the placenta. *Curr Biol* 9, 369-72 (1999)
128. Sugden, P. H.: An overview of endothelin signaling in the cardiac myocyte. *J Mol Cell Cardiol* 35, 871-86 (2003)
129. Nebigil, C. G., N. Etienne, N. Messaddeq & L. Maroteaux: Serotonin is a novel survival factor of cardiomyocytes: mitochondria as a target of 5-HT2B receptor signaling. *Faseb J* 17, 1373-5 (2003)
130. Bueno, O. F., L. J. De Windt, K. M. Tymitz, S. A. Witt, T. R. Kimball, R. Klevitsky, T. E. Hewett, S. P. Jones, D. J. Lefer, C. F. Peng, R. N. Kitsis & J. D. Molkentin: The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *Embo J* 19, 6341-50 (2000)
131. Roovers, K. & R. K. Assoian: Integrating the MAP kinase signal into the G1 phase cell cycle machinery. *Bioessays* 22, 818-26 (2000)
132. Vlahos, C. J., S. A. McDowell & A. Clerk: Kinases as therapeutic targets for heart failure. *Nat Rev Drug Discov* 2, 99-113 (2003)

133. Kohno, M. & J. Pouyssegur: Pharmacological inhibitors of the ERK signaling pathway: application as anticancer drugs. *Prog Cell Cycle Res* 5, 219-24 (2003)
134. Frodin, M. & S. Gammeltoft: Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. *Mol Cell Endocrinol* 151, 65-77 (1999)
135. Sharrocks, A. D.: The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol* 2, 827-37 (2001)
136. Gijon, M. A. & C. C. Leslie: Regulation of arachidonic acid release and cytosolic phospholipase A2 activation. *J Leukoc Biol* 65, 330-6 (1999)
137. Yue, T. L., C. Wang, J. L. Gu, X. L. Ma, S. Kumar, J. C. Lee, G. Z. Feuerstein, H. Thomas, B. Maleeff & E. H. Ohlstein: Inhibition of extracellular signal-regulated kinase enhances Ischemia/Reoxygenation-induced apoptosis in cultured cardiac myocytes and exaggerates reperfusion injury in isolated perfused heart. *Circ Res* 86, 692-9 (2000)
138. Gupta, A., N. S. Aberle, 2nd, R. Kapoor, J. Ren & A. C. Sharma: Bigendothelin-1 via p38-MAPK-dependent mechanism regulates adult rat ventricular myocyte contractility in sepsis. *Biochim Biophys Acta* 1741, 127-39 (2005)
139. Sugden, P. H. & A. Clerk: "Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circ Res* 83, 345-52 (1998)
140. Han, J., J. D. Lee, L. Bibbs & R. J. Ulevitch: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265, 808-11 (1994)
141. Lee, J. C., J. T. Laydon, P. C. McDonnell, T. F. Gallagher, S. Kumar, D. Green, D. McNulty, M. J. Blumenthal, J. R. Heys, S. W. Landvatter & *et al.*: A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372, 739-46 (1994)
142. Jiang, Y., C. Chen, Z. Li, W. Guo, J. A. Gegner, S. Lin & J. Han: Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta). *J Biol Chem* 271, 17920-6 (1996)
143. Kumar, S., P. C. McDonnell, R. J. Gum, A. T. Hand, J. C. Lee & P. R. Young: Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. *Biochem Biophys Res Commun* 235, 533-8 (1997)
144. Li, Z., Y. Jiang, R. J. Ulevitch & J. Han: The primary structure of p38 gamma: a new member of p38 group of MAP kinases. *Biochem Biophys Res Commun* 228, 334-40 (1996)
145. Goedert, M., A. Cuenda, M. Craxton, R. Jakes & P. Cohen: Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases. *Embo J* 16, 3563-71 (1997)
146. Kumar, S., J. Boehm & J. C. Lee: p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. *Nat Rev Drug Discov* 2, 717-26 (2003)
147. Adams, R. H., A. Porras, G. Alonso, M. Jones, K. Vintersten, S. Panelli, A. Valladares, L. Perez, R. Klein & A. R. Nebreda: Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development. *Mol Cell* 6, 109-16 (2000)
148. Chang, L. & M. Karin: Mammalian MAP kinase signalling cascades. *Nature* 410, 37-40 (2001)
149. Lu, H. T., D. D. Yang, M. Wysk, E. Gatti, I. Mellman, R. J. Davis & R. A. Flavell: Defective IL-12 production in mitogen-activated protein (MAP) kinase kinase 3 (Mkk3)-deficient mice. *Embo J* 18, 1845-57 (1999)
150. Ge, B., H. Gram, F. Di Padova, B. Huang, L. New, R. J. Ulevitch, Y. Luo & J. Han: MAPKK-independent activation of p38alpha mediated by TAB1-dependent autophosphorylation of p38alpha. *Science* 295, 1291-4 (2002)
151. Ono, K. & J. Han: The p38 signal transduction pathway: activation and function. *Cell Signal* 12, 1-13 (2000)
152. Petrich, B. G. & Y. Wang: Stress-activated MAP kinases in cardiac remodeling and heart failure; new insights from transgenic studies. *Trends Cardiovasc Med* 14, 50-5 (2004)
153. Adams, J. L., A. M. Badger, S. Kumar & J. C. Lee: p38 MAP kinase: molecular target for the inhibition of pro-inflammatory cytokines. *Prog Med Chem* 38, 1-60 (2001)
154. Ballard-Croft, C., D. J. White, D. L. Maass, D. P. Hybki & J. W. Horton: Role of p38 mitogen-activated protein kinase in cardiac myocyte secretion of the inflammatory cytokine TNF-alpha. *Am J Physiol Heart Circ Physiol* 280, H1970-81 (2001)
155. Kaiser, R. A., O. F. Bueno, D. J. Lips, P. A. Doevendans, F. Jones, T. F. Kimball & J. D. Molkentin: Targeted inhibition of p38 mitogen-activated protein kinase antagonizes cardiac injury and cell death following ischemia-reperfusion *in vivo*. *J Biol Chem* 279, 15524-30 (2004)
156. Mayr, M., Y. Hu, H. Hainaut & Q. Xu: Mechanical stress-induced DNA damage and rac-p38MAPK signal pathways mediate p53-dependent apoptosis in vascular smooth muscle cells. *Faseb J* 16, 1423-5 (2002)
157. Kim, S. J., S. G. Hwang, D. Y. Shin, S. S. Kang & J. S. Chun: p38 kinase regulates nitric oxide-induced apoptosis of articular chondrocytes by accumulating p53 via NFkappa B-dependent transcription and stabilization by serine 15 phosphorylation. *J Biol Chem* 277, 33501-8 (2002)
158. Torcia, M., G. De Chiara, L. Nencioni, S. Ammendola, D. Labardi, M. Lucibello, P. Rosini, L. N. Marlier, P. Bonini, P. Dello Sbarba, A. T. Palamara, N. Zambrano, T. Russo, E. Garaci & F. Cozzolino: Nerve growth factor inhibits apoptosis in memory B lymphocytes via inactivation of p38 MAPK, prevention of Bcl-2 phosphorylation, and cytochrome c release. *J Biol Chem* 276, 39027-36 (2001)
159. Baines, C. P., J. Zhang, G. W. Wang, Y. T. Zheng, J. X. Xiu, E. M. Cardwell, R. Bolli & P. Ping: Mitochondrial PKCepsilon and MAPK form signaling modules in the murine heart: enhanced mitochondrial PKCepsilon-MAPK interactions and differential MAPK activation in PKCepsilon-induced cardioprotection. *Circ Res* 90, 390-7 (2002)
160. Bogoyevitch, M. A., J. Gillespie-Brown, A. J. Ketterman, S. J. Fuller, R. Ben-Levy, A. Ashworth, C. J. Marshall & P. H. Sugden: Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. *Circ Res* 79, 162-73 (1996)
161. Liu, Q. & P. A. Hofmann: Protein phosphatase 2A-mediated cross-talk between p38 MAPK and ERK in

apoptosis of cardiac myocytes. *Am J Physiol Heart Circ Physiol* 286, H2204-12 (2004)
162. Tamura, K., T. Sudo, U. Senftleben, A. M. Dadak, R. Johnson & M. Karin: Requirement for p38alpha in erythropoietin expression: a role for stress kinases in erythropoiesis. *Cell* 102, 221-31 (2000)
163. Cook, S. A., P. H. Sugden & A. Clerk: Activation of c-Jun N-terminal kinases and p38-mitogen-activated protein kinases in human heart failure secondary to ischaemic heart disease. *J Mol Cell Cardiol* 31, 1429-34 (1999)
164. Zechner, D., D. J. Thuerauf, D. S. Hanford, P. M. McDonough & C. C. Glembotski: A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. *J Cell Biol* 139, 115-27 (1997)
165. Braz, J. C., O. F. Bueno, Q. Liang, B. J. Wilkins, Y. S. Dai, S. Parsons, J. Braunwart, B. J. Glascock, R. Klevitsky, T. F. Kimball, T. E. Hewett & J. D. Molkentin: Targeted inhibition of p38 MAPK promotes hypertrophic cardiomyopathy through upregulation of calcineurin-NFAT signaling. *J Clin Invest* 111, 1475-86 (2003)
166. Zhang, S., C. Weinheimer, M. Courtois, A. Kovacs, C. E. Zhang, A. M. Cheng, Y. Wang & A. J. Muslin: The role of the Grb2-p38 MAPK signaling pathway in cardiac hypertrophy and fibrosis. *J Clin Invest* 111, 833-41 (2003)
167. Behr, T. M., S. S. Nerurkar, A. H. Nelson, R. W. Coatney, T. N. Woods, A. Sulpizio, S. Chandra, D. P. Brooks, S. Kumar, J. C. Lee, E. H. Ohlstein, C. E. Angermann, J. L. Adams, J. Sisko, J. D. Sackner-Bernstein & R. N. Willette: Hypertensive end-organ damage and premature mortality are p38 mitogen-activated protein kinase-dependent in a rat model of cardiac hypertrophy and dysfunction. *Circulation* 104, 1292-8 (2001)
168. Liao, P., S. Q. Wang, S. Wang, M. Zheng, M. Zheng, S. J. Zhang, H. Cheng, Y. Wang & R. P. Xiao: p38 Mitogen-activated protein kinase mediates a negative inotropic effect in cardiac myocytes. *Circ Res* 90, 190-6 (2002)
169. Liao, P., D. Georgakopoulos, A. Kovacs, M. Zheng, D. Lerner, H. Pu, J. Saffitz, K. Chien, R. P. Xiao, D. A. Kass & Y. Wang: The *in vivo* role of p38 MAP kinases in cardiac remodeling and restrictive cardiomyopathy. *Proc Natl Acad Sci U S A* 98, 12283-8 (2001)
170. Degousee, N., J. Martindale, E. Stefanski, M. Cieslak, T. F. Lindsay, J. E. Fish, P. A. Marsden, D. J. Thuerauf, C. C. Glembotski & B. B. Rubin: MAP kinase kinase 6-p38 MAP kinase signaling cascade regulates cyclooxygenase-2 expression in cardiac myocytes *in vitro* and *in vivo*. *Circ Res* 92, 757-64 (2003)
171. Rebsamen, M. C., R. Capoccia, M. B. Vallotton & U. Lang: Role of cyclooxygenase 2, p38 and p42/44 MAPK in the secretion of prostacyclin induced by epidermal growth factor, endothelin-1 and angiotensin II in rat ventricular cardiomyocytes. *J Mol Cell Cardiol* 35, 81-9 (2003)
172. Manning, G., D. B. Whyte, R. Martinez, T. Hunter & S. Sudarsanam: The protein kinase complement of the human genome. *Science* 298, 1912-34 (2002)
173. Yamazaki, T., H. Kurihara, Y. Kurihara, I. Komuro & Y. Yazaki: Endothelin-1 regulates normal cardiovascular development and cardiac cellular hypertrophy. *J Card Fail* 2, S7-12 (1996)
174. Gupta, S., T. Barrett, A. J. Whitmarsh, J. Cavanagh, H. K. Sluss, B. Derijard & R. J. Davis: Selective interaction of JNK protein kinase isoforms with transcription factors. *Embo J* 15, 2760-70 (1996)
175. Kuan, C. Y., D. D. Yang, D. R. Samanta Roy, R. J. Davis, P. Rakic & R. A. Flavell: The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* 22, 667-76 (1999)
176. Su, B., E. Jacinto, M. Hibi, T. Kallunki, M. Karin & Y. Ben-Neriah: JNK is involved in signal integration during costimulation of T lymphocytes. *Cell* 77, 727-36 (1994)
177. Wang, Y., B. Su, V. P. Sah, J. H. Brown, J. Han & K. R. Chien: Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH2-terminal kinase in ventricular muscle cells. *J Biol Chem* 273, 5423-6 (1998)
178. Choukroun, G., R. Hajjar, J. M. Kyriakis, J. V. Bonventre, A. Rosenzweig & T. Force: Role of the stress-activated protein kinases in endothelin-induced cardiomyocyte hypertrophy. *J Clin Invest* 102, 1311-20 (1998)
179. Thorburn, J., S. Xu & A. Thorburn: MAP kinase- and Rho-dependent signals interact to regulate gene expression but not actin morphology in cardiac muscle cells. *Embo J* 16, 1888-900 (1997)
180. Liang, Q., O. F. Bueno, B. J. Wilkins, C. Y. Kuan, Y. Xia & J. D. Molkentin: c-Jun N-terminal kinases (JNK) antagonize cardiac growth through cross-talk with calcineurin-NFAT signaling. *Embo J* 22, 5079-89 (2003)
181. Izumiya, Y., S. Kim, Y. Izumi, K. Yoshida, M. Yoshiyama, A. Matsuzawa, H. Ichijo & H. Iwao: Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II-induced cardiac hypertrophy and remodeling. *Circ Res* 93, 874-83 (2003)
182. Sadoshima, J., O. Montagne, Q. Wang, G. Yang, J. Warden, J. Liu, G. Takagi, V. Karoor, C. Hong, G. L. Johnson, D. E. Vatner & S. F. Vatner: The MEKK1-JNK pathway plays a protective role in pressure overload but does not mediate cardiac hypertrophy. *J Clin Invest* 110, 271-9 (2002)
183. Hreniuk, D., M. Garay, W. Gaarde, B. P. Monia, R. A. McKay & C. L. Cioffi: Inhibition of c-Jun N-terminal kinase 1, but not c-Jun N-terminal kinase 2, suppresses apoptosis induced by ischemia/reoxygenation in rat cardiac myocytes. *Mol Pharmacol* 59, 867-74 (2001)
184. Aoki, H., P. M. Kang, J. Hampe, K. Yoshimura, T. Noma, M. Matsuzaki & S. Izumo: Direct activation of mitochondrial apoptosis machinery by c-Jun N-terminal kinase in adult cardiac myocytes. *J Biol Chem* 277, 10244-50 (2002)
185. Shaulian, E., M. Schreiber, F. Piu, M. Beeche, E. F. Wagner & M. Karin: The mammalian UV response: c-Jun induction is required for exit from p53-imposed growth arrest. *Cell* 103, 897-907 (2000)
186. Andreka, P., J. Zang, C. Dougherty, T. I. Slepak, K. A. Webster & N. H. Bishopric: Cytoprotection by Jun kinase during nitric oxide-induced cardiac myocyte apoptosis. *Circ Res* 88, 305-12 (2001)
187. Ashkenazi, A. & V. M. Dixit: Death receptors: signaling and modulation. *Science* 281, 1305-8 (1998)
188. Hengartner, M. O.: The biochemistry of apoptosis. *Nature* 407, 770-6 (2000)

189. Krammer, P. H.: CD95's deadly mission in the immune system. *Nature* 407, 789-95 (2000)
190. Bratton, S. B., J. Lewis, M. Butterworth, C. S. Duckett & G. M. Cohen: XIAP inhibition of caspase-3 preserves its association with the Apaf-1 apoptosome and prevents CD95- and Bax-induced apoptosis. *Cell Death Differ* 9, 881-92 (2002)
191. Bratton, S. B., M. MacFarlane, K. Cain & G. M. Cohen: Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. *Exp Cell Res* 256, 27-33 (2000)
192. Green, D. R. & J. C. Reed: Mitochondria and apoptosis. *Science* 281, 1309-12 (1998)
193. Cain, K., D. G. Brown, C. Langlais & G. M. Cohen: Caspase activation involves the formation of the aposome, a large (approximately 700 kDa) caspase-activating complex. *J Biol Chem* 274, 22686-92 (1999)
194. Cain, K., S. B. Bratton, C. Langlais, G. Walker, D. G. Brown, X. M. Sun & G. M. Cohen: Apaf-1 oligomerizes into biologically active approximately 700-kDa and inactive approximately 1.4-MDa apoptosome complexes. *J Biol Chem* 275, 6067-70 (2000)
195. Zou, H., Y. Li, X. Liu & X. Wang: An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem* 274, 11549-56 (1999)
196. Harris, M. H. & C. B. Thompson: The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. *Cell Death Differ* 7, 1182-91 (2000)
197. Gonzalez, A., M. A. Fortuno, R. Querejeta, S. Ravassa, B. Lopez, N. Lopez & J. Diez: Cardiomyocyte apoptosis in hypertensive cardiomyopathy. *Cardiovasc Res* 59, 549-62 (2003)
198. Matsuzawa, A. & H. Ichijo: Molecular mechanisms of the decision between life and death: regulation of apoptosis by apoptosis signal-regulating kinase 1. *J Biochem (Tokyo)* 130, 1-8 (2001)
199. Livingstone, C., G. Patel & N. Jones: ATF-2 contains a phosphorylation-dependent transcriptional activation domain. *Embo J* 14, 1785-97 (1995)
200. Hein, S., E. Arnon, S. Kostin, M. Schonburg, A. Elsasser, V. Polyakova, E. P. Bauer, W. P. Klovekorn & J. Schaper: Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 107, 984-91 (2003)
201. Crabtree, G. R.: Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. *Cell* 96, 611-4 (1999)
202. Klee, C. B., H. Ren & X. Wang: Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. *J Biol Chem* 273, 13367-70 (1998)
203. Dolmetsch, R. E., R. S. Lewis, C. C. Goodnow & J. I. Healy: Differential activation of transcription factors induced by Ca2+ response amplitude and duration. *Nature* 386, 855-8 (1997)
204. Wilkins, B. J. & J. D. Molkentin: Calcineurin and cardiac hypertrophy: where have we been? Where are we going? *J Physiol* 541, 1-8 (2002)
205. Kim, Y., J. S. Moon, K. S. Lee, S. Y. Park, J. Cheong, H. S. Kang, H. Y. Lee & H. D. Kim: Ca2+/calmodulin-dependent protein phosphatase calcineurin mediates the expression of iNOS through IKK and NF-kappaB activity in LPS-stimulated mouse peritoneal macrophages and RAW 264.7 cells. *Biochem Biophys Res Commun* 314, 695-703 (2004)
206. Newton, A. C.: Protein kinase C: structure, function, and regulation. *J Biol Chem* 270, 28495-8 (1995)
207. Pass, J. M., J. Gao, W. K. Jones, W. B. Wead, X. Wu, J. Zhang, C. P. Baines, R. Bolli, Y. T. Zheng, I. G. Joshua & P. Ping: Enhanced PKC beta II translocation and PKC beta II-RACK1 interactions in PKC epsilon-induced heart failure: a role for RACK1. *Am J Physiol Heart Circ Physiol* 281, H2500-10 (2001)
208. Ping, P., J. Zhang, Y. Qiu, X. L. Tang, S. Manchikalapudi, X. Cao & R. Bolli: Ischemic preconditioning induces selective translocation of protein kinase C isoforms epsilon and eta in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. *Circ Res* 81, 404-14 (1997)
209. Balafanova, Z., R. Bolli, J. Zhang, Y. Zheng, J. M. Pass, A. Bhatnagar, X. L. Tang, O. Wang, E. Cardwell & P. Ping: Nitric oxide (NO) induces nitration of protein kinase Cepsilon (PKCepsilon), facilitating PKCepsilon translocation via enhanced PKCepsilon -RACK2 interactions: a novel mechanism of no-triggered activation of PKCepsilon. *J Biol Chem* 277, 15021-7 (2002)
210. Disatnik, M. H., S. N. Jones & D. Mochly-Rosen: Stimulus-dependent subcellular localization of activated protein kinase C; a study with acidic fibroblast growth factor and transforming growth factor-beta 1 in cardiac myocytes. *J Mol Cell Cardiol* 27, 2473-81 (1995)
211. Takeishi, Y., T. Jalili, N. A. Ball & R. A. Walsh: Responses of cardiac protein kinase C isoforms to distinct pathological stimuli are differentially regulated. *Circ Res* 85, 264-71 (1999)
212. Heidkamp, M. C., A. L. Bayer, J. L. Martin & A. M. Samarel: Differential activation of mitogen-activated protein kinase cascades and apoptosis by protein kinase C epsilon and delta in neonatal rat ventricular myocytes. *Circ Res* 89, 882-90 (2001)
213. Zhou, H. Z., J. S. Karliner & M. O. Gray: Moderate alcohol consumption induces sustained cardiac protection by activating PKC-epsilon and Akt. *Am J Physiol Heart Circ Physiol* 283, H165-74 (2002)
214. Brodie, C. & P. M. Blumberg: Regulation of cell apoptosis by protein kinase c delta. *Apoptosis* 8, 19-27 (2003)
215. Kim, J. S., Y. Jin & J. J. Lemasters: Inhibition of protein kinase kinase C delta prevents the mitochondrial permeability transition- and pH-dependent killing in cultured adult rat myocytes after ischemia/reperfusion. *Circulation* 108, 220 (Abstr) (2003)
216. Majumder, P. K., P. Pandey, X. Sun, K. Cheng, R. Datta, S. Saxena, S. Kharbanda & D. Kufe: Mitochondrial translocation of protein kinase C delta in phorbol ester-induced cytochrome c release and apoptosis. *J Biol Chem* 275, 21793-6 (2000)
217. Li, L., P. S. Lorenzo, K. Bogi, P. M. Blumberg & S. H. Yuspa: Protein kinase Cdelta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. *Mol Cell Biol* 19, 8547-58 (1999)

218. McJilton, M. A., C. Van Sikes, G. G. Wescott, D. Wu, T. L. Foreman, C. W. Gregory, D. A. Weidner, O. Harris Ford, A. Morgan Lasater, J. L. Mohler & D. M. Terrian: Protein kinase Cepsilon interacts with Bax and promotes survival of human prostate cancer cells. *Oncogene* 22, 7958-68 (2003)
219. Ding, L., H. Wang, W. Lang & L. Xiao: Protein kinase C-epsilon promotes survival of lung cancer cells by suppressing apoptosis through dysregulation of the mitochondrial caspase pathway. *J Biol Chem* 277, 35305-13 (2002)
220. Gupta, A. & A. C. Sharma: Metalloendopeptidase inhibition regulates phosphorylation of p38-mitogen-activated protein kinase and nitric oxide synthase in heart after endotoxemia. *Shock* 20, 375-81 (2003)

Key Words: Inflammation, Infection, Sepsis, Systemic Inflammatory Response Syndrome, Peritonitis, Polymicrobial sepsis, *tau,* Apoptosis, Review

Send correspondence to: Avadhesh C Sharma, PharmD, PhD, FAHA, Cardionome Laboratory, Department of Pharmaceutical Sciences, North Dakota State University, 208 Sudro Hall, Fargo, ND 58105, Tel: 701-231-7780, Fax: 701-231-8333, E-mail: Avadhesh.sharma@ndsu.edu