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Cytokine Profile of Human Septic Shock Serum Inducing Cardiomyocyte Contractile Dysfunction

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Summary

This study was designed to measure nitrite/nitrate and cytokine levels of serum obtained from septic shock patients and to describe potential depressant effects of human septic serum on rat cardiomyocytes. Serum was prepared from 10 non-septic patients and 10 patients with documented septic shock. Adult rat ventricular myocytes were exposed to 20 % serum in the medium. Cardiomyocyte contractility was assessed by measuring shortening fraction and shortening velocity. Serum levels of nitrite/nitrate, a marker of nitric oxide final metabolites, and cytokines (tumor necrosis factor (TNF)-α, interleukin (IL) 1β, 6, 10, 8 and 12p70) were measured. Compared with serum from non-septic patients, serum of septic shock patients induced rapid reduction of the extent and velocity of shortening in isolated cardiomyocytes. Nitrite/nitrate, TNF-α, IL-1β and IL-12p70 concentrations of tested serum for cardiomyocyte studies were not increased in septic serum compared with controls. In contrast, septic serum that induced a depression of *in vitro* contractility, had increased levels of IL-6, IL-8 and IL-10. We can conclude that the depression of *in vitro* contractility induced by septic serum is not directly dependent on elevated levels of nitric oxide metabolites, TNF-α or IL-1β. Our results support the view that other cytokines, including IL-6, IL-8 and IL-10, are potent circulating mediators of myocardial depression in cardiomyocytes.

Key words
Heart dysfunction • Tumor necrosis factor • Interleukin • Sepsis • Cell shortening

Introduction

Endotoxin, or bacterial lipopolysaccharide, elicits a cascade of pro and anti-inflammatory cytokine responses which may induce myocardial contractility depression both in laboratory animals and human sepsis. In this context, myocardial dysfunction is a consistent and important feature of septic shock and contributes to the high mortality rate associated with this disorder (Court *et al*. 2002, Krishnagopalan *et al*. 2002). Although sepsis-induced myocardial depression is a well defined entity in the clinical literature, the cellular basis for this reduction in contractility is still poorly understood. Myocardial dysfunction does not appear to be due to myocardial hypoperfusion (Kumar *et al*. 2000) but rather due to circulating depressant factors, including cytokine

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tumor necrosis factor (TNF)-α and interleukin (IL)-1β (Parrillo *et al*. 1985, Kumar *et al*. 2003). At a cellular level, reduced myocardial contractility seems to be induced by both nitric oxide-dependent and nitric oxide-independent mechanisms. Indeed, TNF-α and IL-1β alter myocardial function *via* a cascade of intracellular events, which includes activation of neutral sphingomyelinase and nitric oxide pathways, suppression of the calcium transient, mitochondrial dysfunction, and apoptosis (Parker 1998, Levy and Deutschman 2004).

During human septic shock, circulating myocardial depressant substances that directly alter *in vitro* cardiomyocyte contractility has been described (Lefer 1979, Parrillo *et al*. 1985, Kumar *et al*. 1996, Pathan *et al*. 2002, 2004) . For example, elevated serum concentrations of TNF-α (alone or in association with IL-1β) (Lefer 1979, Pathan *et al*. 2002, 2004) and nitric oxide metabolites (Parker 1998, Levy and Deutschman 2004) that cause cardiomyocyte depression *in vitro* are found during human sepsis. In addition, removal of TNF-α by monoclonal antibodies and immunoabsorption of IL-1β partially neutralizes cardiac myocyte depressant activity of human septic serum. However, extensive characterization of multiple cytokine concentrations and pathological production of nitric oxide of septic serum that induces *in vitro* cardiac depression are not readily available.

The major goal of our study was to test whether septic serum that induces reductions in cardiomyocyte contractile function has elevated pro- and anti-inflammatory cytokine concentrations and nitrite/nitrate levels. Altogether our results show that septic serum which induced major reductions in cardiomyocyte cell shortening had no increased levels of TNF-α, IL-1β and nitrite/nitrate. In sharp contrast, IL-6, IL-8 and IL-10 levels were increased in septic serum compared with the control serum.

Methods

Patients

After ethical committee approval and written informed consent from the legal representatives, 10 patients suffering from septic shock along with 10 control patients with non-septic chronic obstructive pulmonary disease were enrolled in this pilot study. Patients fulfilled the clinical and laboratory criteria of septic shock as outlined in the 1992 Consensus Conference. Exclusion criteria were age <18 years, pregnancy, patients who have had surgery within 48 h before inclusion and patients who have had cardiac surgery and neurosurgery. Patients with an acute history of severe cardiac insufficiency (New York Heart Association class III-IV) and coronary artery disease before the development of septic shock were also excluded. The rationale for this was to omit myocardial congestive failure as a confounding factor of serum-induced cardiomyocyte contractile depression.

On intensive care unit (ICU) admission, standard volume resuscitation combined with inotropic/vasopressor support was used to maintain the systolic arterial pressure above 90 mm Hg. All patients received mechanical ventilation. Assessment of left ventricular (LV) systolic function by echocardiography revealed a markedly hypokinetic LV (mean LV ejection fraction: 39±17 %). Blood smples were withdrawn within the first hours after patient ICU admission. Venous blood was collected on admission into a 10 ml sterile plain tube before administration of any medications and stored at –80 °C until use. Before assay, all samples were thawed to room temperature and mixed by gentle swirling. All serum samples were assayed on the same day to avoid inter-assay variation.

Biological assays for nitrite/nitrate and cytokine concentrations

Nitrite/nitrate levels, an indicator of nitric oxide (NO) synthesis, were measured. First, nitrate in serum were reduced to nitrite by adding nitrate reductase (25 mU/ml; Sigma, Saint Quentin Fallavier, France) and NADPH (200 μM; Calbiochem) at room temperature. After 3 h, samples were deproteinized by adding a solution of $ZnSO_4$ 30 %, and 15 min later, samples were centrifuged at 2000 xg for 10 min. Nitrite concentration in the samples was measured by the Griess reaction: 100 μl of Griess reagent (0.1 % naphthalethylenediamine dihydrochloride in H_2O and 1 % sulfanilamide in 5 % concentrated H_3PO_4; vol 1:1; Molecular Probes, Eugene, Oregon, USA) were added to 100 μl of supernatants. The optical density at 550 nm (OD 550) was measured using a microplate reader. Nitrate concentrations were calculated by comparison with OD 550 of standard solutions of sodium nitrite (Lancel *et al*. 2004).

Multiplex bead kits (Human Iinflammation Kit, BD™ Cytometric Bead Array were purchased from Becton Dickinson Company, Franklin Lakes, USA). Cytokines analyzed in duplicate by each kit included tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10

(IL-10) and interleukin-12 (IL-12p70). Each multiplex assay was performed in duplicate according to the manufacturers' specifications. Standard curves for each cytokine were generated by using the reference cytokine concentrations supplied by the manufacturer. To obtain concentration values, raw data (mean fluorescent intensity) were analyzed as recommended by BD™ Cytometric Bead Array, Becton Dickinson Company.

Left ventricle cardiomyocyte contractile function

Rat experiments were conducted in accordance with the National and European Institutes of Health guidelines for the use of laboratory animals and were approved by Lille University Ethic Committee. Freshly isolated cells were prepared from ventricles of Sprague-Dawley rats (Lancel *et al*. 2005). Hearts were perfused through the aorta for 5 min with a nominally calcium-free modified Krebs-Henseleit (KH) buffer (in mmol/l: NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 11) after which the buffer was changed to one containing 0.1 % of collagenase type II (Worthington, Lakewood, NJ, USA) and 0.01 % of protease type XIV (Sigma St, Quentin Fallavier, France). The cells were separated on a Percoll gradient and plated in dishes to a concentration of 10^5 cells/ml. Cells were stored at 37 °C and used within 3 h after isolation. Cell viability (> 85 %) was assessed by the Trypan blue exclusion test on about 100 cells.

Primary cultured adult rat ventricular myocytes were placed in the perfusion chamber (Krebs-Henseleit Ca^{2+} 1.8 mmol/l) on the thermo regulated (30 °C) stage of a Nikon Eclipse E 800 microscope (Nikon, Paris, France) and then stimulated to contract (5 ms square pulse, voltage-adjusted to maximize capture) at 1 Hz (Grass S48, Grass Instrument Division, Astro-Med, Trappes, France). The steady-state contractions were recorded and digitized at a rate of 256 Hz. Edge-detection algorithms allowed derivation of contractile parameters (IonOptix, Milton, MA, USA). Measurements from 12 steady-state contractions were averaged for each cell (Lancel *et al.* 2005). Primary cultured adult rat ventricular myocytes were incubated in standard growth media consisted of Medium 199 (calcium 1.8 mM) (GIBCO Laboratories, Grand Island, NY) supplemented with insulin (10 mg/ml), L-carnitine (20 mM), creatine (50 mM), taurine (50 mM), penicillin and streptomycin (Sigma, St. Quentin Fallavier, France). Test media used 20 % human serum of either control or septic shock patients. Measurements of maximum extent and peak velocity of cardiomyocyte shortening were obtained every 5 min for 60 min.

Statistical analysis

By comparing the maximum extent and peak velocity of shortening at each 10-min interval to the baseline value, changes were compared to initial contractility. Data for the change in maximum extent and peak velocity of cardiomyocyte shortening (percentage change from baseline) were pooled and plotted as a function of time for each control and septic shock serum. Linear regression analysis was utilized to fit a line for each resulting plot. Slopes of lines for control and septic shock serum were compared by a two-tailed Student's t-test to determine whether these slopes were significantly different. In this manner, increased depressant activity was indicated by a more negative value for slope of the regression line (Kumar *et al*. 1999). All other analyses used ANOVA procedures where appropriate. When a significant difference was found, we identified specific differences between groups using a sequentially rejective Bonferroni procedure. After application of the Bonferroni correction, significance was evaluated with $P<0.05$ for comparisons.

Results

Ten septic shock patients were included with the diagnosis of peritonitis (4 patients), pneumonia (4 patients) and soft tissue infection (2 patients). Severity of disease was measured by Acute Physiology and Chronic Health Evaluation (APACHE) II and Sepsis-related Organ Failure Assessment (SOFA) scores at admittance, averaging 22±7 and 10.3±4.7, respectively. Ten non-septic patients with no signs of sepsis served as controls. Non septic patients were admitted in ICU for acute respiratory failure in the context of chronic obstructive pulmonary disease (APACHE II score 17±9).

Changes in maximum extent and velocity of myocardial cell shortening as a function of time after exposure to either 20 % control or septic shock patient serum are presented in Figures 1 and 2. In each case, 20 % serum from septic shock patients caused significant depression of both extent (Fig. 1) and velocity (Fig. 2) of shortening compared with controls.

On the basis of week to week analysis using different LV cardiomyocyte isolations, we confirmed that control serum induced minimal cardiomyocyte contractile effects whereas septic shock serum consistently reduced

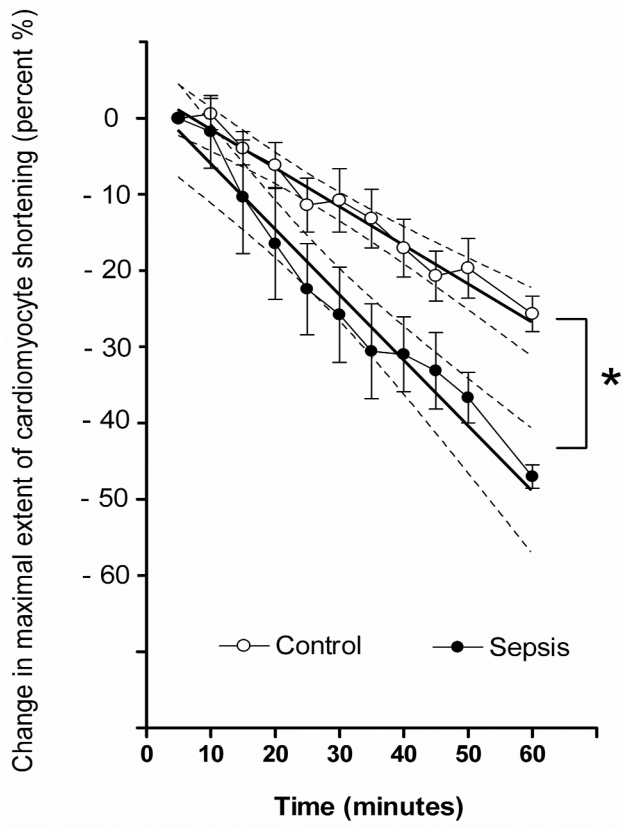

Fig. 1. Changes in maximum extent of myocardial cell shortening as a function of time. Data for the change in maximum extent cardiomyocyte shortening (percentage change from baseline) are pooled and plotted as a function of time after incubation with 20 % serum of control patient (n=10) and septic shock patients (n=10). Linear regression analysis was used to fit a line for each resulting plot. Slopes of changes for serum of control and septic shock patients were statistically different by two-tailed Student's t-test; * P<0.01. Data are means ± S.D.

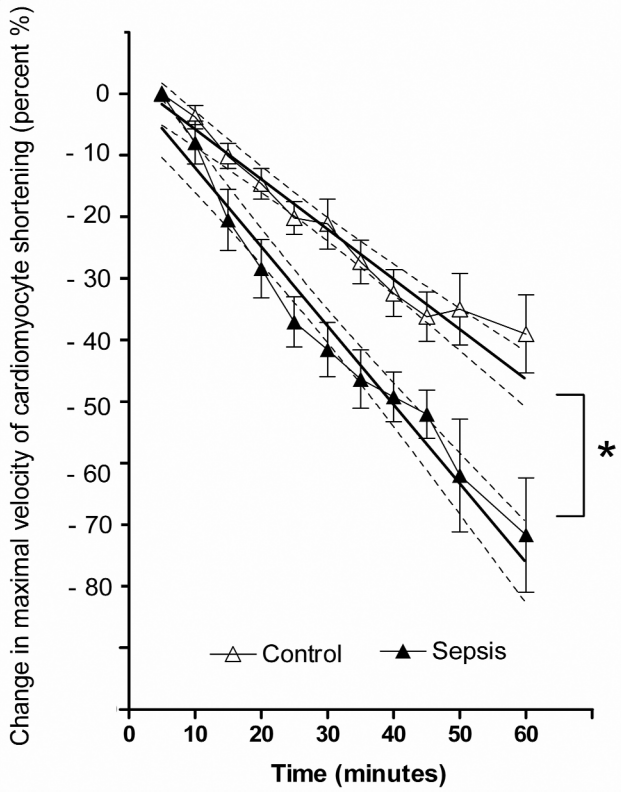

Fig. 2. Changes in maximum velocity of myocardial cell shortening as a function of time. Data for the change in maximum velocity cardiomyocyte shortening (percentage change from baseline) are pooled and plotted as a function of time after incubation with 20 % serum of control patient (n=10) and septic shock patients (n=10). Linear regression analysis was used to fit a line for each resulting plot. Slopes of changes for serum of control and septic shock patients were statistically different by two-tailed Student's t-test; * P<0.01. Data are means ± S.D.

contractile function (data not shown).

There were no differences in nitrite/nitrate levels between serum of controls and septic shock patients (22±8 vs 24±12 μmol/l). Serum cytokine concentrations of controls and septic shock patients are presented in Figure 3. We mainly observed that in septic serum, inducing reductions in cardiomyocyte cell shortening, IL-6, IL-8 and IL-10 levels were increased compared with control serum. Control and septic serum had similar concentrations of TNF-α and IL-1β.

Discussion

The objective of our study was to test whether septic sera that induce reductions in cardiomyocyte contractile function has elevated pro and anti-inflammatory cytokine and nitrite/nitrate concentrations. Our results indicate that the septic serum, which induced reductions in cardiomyocyte cell shortening, had no increased levels of TNF-α, IL-1β and nitrite/nitrate. On the contrary, IL-6, IL-8, and IL-10 levels in septic serum were increased, which could play a role in septic serum-induced cardiomyocyte cell shortening depression. Although no direct link between cardiomyocyte contractile function and serum cytokine levels was tested, we believe that our experiments are important for designing further studies of the role of cytokines such as IL-6, IL-8 and IL-10 in the pathophysiology of endotoxin-induced myocardial depression.

A wide range of circulating inflammatory mediators have been reported to induce myocardial depressant activity both in isolated cardiomyocytes and in the whole heart (Natanson *et al*. 1989, Kapadia *et al*. 1995). These include proinflammatory cytokines and nitric oxide pathway metabolites (Kumar *et al*. 2000, 2003, Court *et al*. 2002, Krishnagopalan *et al*. 2002). Sizing of the circulating myocardial depressant factor estimated by gel filtration suggests a molecular weight of

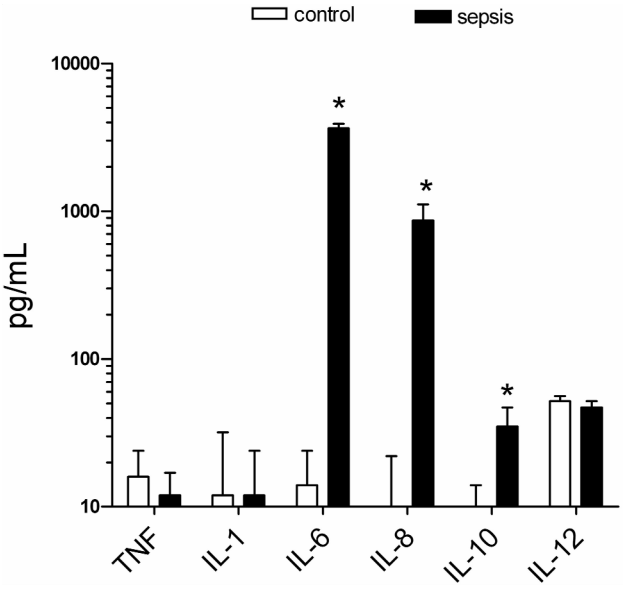

Fig. 3 Cytokine concentrations in serum of control and septic shock patients. Concentrations of tumor necrosis factor-α (TNF), interleukin-1β (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and interleukin-12 p70 (IL-12) were measured in the serum by the use of multiplex assay. Y axis is given in $\log_{10}$ cytokine concentrations. Results were analyzed by ANOVA procedures. After application of Bonferroni correction, significance was achieved with * $P < 0.01$ for comparisons. Data are means ± S.D.

10–25 kDa, similar to the expected molecular weight of TNF-α (17 kDa) and other proinflammatory cytokines including IL-1β (Parrillo *et al*. 1985, Reilly *et al*. 1989, Kumar *et al*. 1999, Pathan *et al*. 2002, 2004, Kumar *et al*. 2003). Negative inotropic role of TNF-α and IL-1β has been strengthened by the evidence that removal of TNF-α by monoclonal antibodies and immunoadsorption of IL-1β partially neutralize cardiomyocyte depressant activity of human septic serum (Parrillo *et al*. 1985). Results from previous studies strongly suggest that TNF-α alone or in association with IL-1β is critical for septic serum-induced cardiodepression. However, it should be noted that no extensive information on cytokine concentrations and other potent depressor mediators present in human septic serum was available in the above cited studies.

To the best of our knowledge, the present study is the first to report multiple cytokine and nitrite/nitrate concentrations in septic shock serum, which actually induces major reductions in cardiomyocyte contractile function. Surprisingly, there were no differences in TNF-α, IL-1β and nitrite/nitrate levels between control and septic shock serum. These results raise the question of their implication in the observed reduction of contractile function of cardiomyocytes induced by septic serum. Indeed, the effects of TNF-α and IL-1β on myocardial function are not consistent with reports showing either increases, decreases or even no effects on cardiomyocyte contractile function (Finkel *et al*. 1992, Amadou *et al*. 2002, Cailleret *et al*. 2004, Pathan *et al*. 2004). Hence, other cytokines within sizing of myocardial depressant factor(s) could be implicated. For example, we found that concentration of IL-6 was markedly increased in serum of septic shock patients. Elevated serum IL-6 concentration could be responsible, at least in part, for myocardial depression as IL-6 has been shown to depress papillary muscle contraction and is negatively inotropic in cardiomyocyte cultures (Finkel *et al*. 1992, Kinugawa *et al*. 1994). In addition, myocardial depressant activity of serum from patients with meningococcemia is completely removed by affinity-adsorption of interleukin 6, while immunoadsorption of TNF-α had no effects (Pathan *et al*. 2004). IL-6-induced decrease in cardiac contractility is mediated by rapid (5-10 min) activation of Janus kinase (JAK)2/signal transducers and activators of transcription (STAT)3 leading to nitric oxide production, reduced sarcoplasmic reticular function and concomitant decrease in the phosphorylation of phospholamban (Yu *et al*. 2005a,b).

Previous results suggested that IL-8 and IL-10 may be homeostatic regulators of hemodynamic parameters, leukocyte-endothelial cell interactions, and microvascular dysfunction in sepsis. Our results reveal that concentrations of IL-8 and IL-10 were markedly increased in the serum of septic shock patients and negatively alter cardiomyocyte contractile function. At this time, controversy exists over the role of these interleukins in sepsis. For example, IL-10 is considered to have both anti-inflammatory and immune suppressive effects and may be protective in models of endotoxemia whereas it seems to be deleterious in models of polymicrobial sepsis (Parsons 1998).

Several reports have linked increased production of the endogenous vasodilator nitric oxide to the occurrence of myocardial dysfunction during experimental septic shock (Kumar *et al*. 2000, Court *et al*. 2002, Krishnagopalan *et al*. 2002). Further evidence for a role of nitric oxide in septic shock is derived from several studies in humans showing elevated plasma concentrations of the nitric oxide bioreaction products, i.e. nitrite/nitrate (Gomez-Jimenez *et al*. 1995, De Werra *et al*. 1997, Strand *et al*. 2000, Mitaka *et al*. 2003, Soop *et al*. 2003). Although several studies of nitrite/nitrate plasma concentrations in human sepsis have showed elevated levels, a substantial fraction of patients with

septic shock and human volunteers exposed to endotoxin has plasma nitrite/nitrate concentrations within the normal range. In the present study, there were no differences in nitrite/nitrate levels between serum of controls and septic shock patients. Hence, based on the available literature and our own results, the hypothesis that nitric oxide metabolites are central mediators in cardiomyocyte dysfunction induced by the serum of septic shock patients can thus be questioned.

Several limitations may be found in our study. First, myocardial dysfunction in septic shock patients was established on the basis of non-invasive echocardiographic assessment. Only major reductions in left ventricular ejection fraction were used to define myocardial dysfunction. No correlation between reductions in left ventricular ejection fraction and effects of serum on cardiomyocyte contractile function was tested. Results from our study are only descriptive and no attempt was made to find a link between cardiomyocyte contractile function and serum cytokine levels has been achieved. However, our study supports the view that cytokine(s) other than TNF-α and IL-1β may be implicated in septic serum-induced cardiomyocyte contractile depression. Based on the analysis of a cytokine panel that measures cytokine wide ranges, we choose to implicate IL-6 in myocardial depression in sepsis as this cytokine has been recently shown to impact heart function. Endogenous vasodilator nitric oxide has been linked to the occurrence of myocardial dysfunction during septic shock. Pathological production of nitric oxide was assessed by the means of nitrite/nitrate levels that may not accurately reflect nitric oxide metabolism *in vivo*. Also, cardiac toxicity of peroxynitrite, a reactive nitrogen species produced in presence of reactive oxygen species and nitric oxide, has not been directly tested herein.

In conclusion, results from our and other studies support to the view that interleukin 6 as a potent circulating mediator of myocardial depression in cardiomyocytes. Further efforts should be made to identify interleukin IL-6 is a plausible cause of myocardial dysfunction in sepsis.

References

AMADOU A, NAWROCKI A, BEST-BELPOMME M, PAVOINE C, PECKER F: Arachidonic acid mediates dual effect of TNF-alpha on Ca^{2+} transients and contraction of adult rat cardiomyocytes. *Am J Physiol* **282**: C1339-C1347, 2002.

CAILLERET M, AMADOU A, ANDRIEU-ABADIE N, NAWROCKI A, ADAMY C, AIT-MAMAR B, ROCARIES F, BEST-BELPOMME M, LEVADE T, PAVOINE C, PECKER F: N-acetylcysteine prevents the deleterious effect of tumor necrosis factor-(alpha) on calcium transients and contraction in adult rat cardiomyocytes. *Circulation* **109**: 406-411, 2004.

COURT O, KUMAR A, PARRILLO JE, KUMAR A: Clinical review: Myocardial depression in sepsis and septic shock. *Crit Care* **6**: 500-508, 2002.

DE WERRA I, JACCARD C, CORRADIN SB, CHIOLERO R, YERSIN B, GALLATI H, ASSICOT M, BOHUON C, BAUMGARTNER JD, GLAUSER MP, HEUMANN D: Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. *Crit Care Med* **25**: 607-613, 1997.

FINKEL MS, ODDIS CV, JACOB TD, WATKINS SC, HATTLER BG, SIMMONS RL: Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* **257**: 387-389, 1992.

GOMEZ-JIMENEZ J, SALGADO A, MOURELLE, MARTIN MC, SEGURA RM, PERACAULA R, MONCADA S: L-arginine: nitric oxide pathway in endotoxemia and human septic shock. *Crit Care Med* **23**: 253-258, 1995.

KAPADIA S, LEE J, TORRE-AMIONE G, BIRDSALL HH, MA TS, MANN DL: Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. *J Clin Invest* **96**: 1042-1052, 1995.

KINUGAWA K, TAKAHASHI T, KOHMOTO O, KOHMOTO O, SERIZAWA T, TAKAHASHI T: Nitric oxide-mediated effects of interleukin-6 on $[Ca^{2+}]i$ and cell contraction in cultured chick ventricular myocytes. *Circ Res* **75**: 285-295, 1994.

KRISHNAGOPALAN S, KUMAR A, PARRILLO JE, KUMAR A: Myocardial dysfunction in the patient with sepsis. *Curr Opin Crit Care* **8**: 376-388, 2002.

KUMAR A, THOTA V, DEE L, OLSON J, URETZ E, PARRILLO JE: Tumor necrosis factor α and interleukin 1β are responsible for in vitro myocardial cell depression induced by human septic shock serum. *J Exp Med* **183**: 949-958, 1996.

KUMAR A, BRAR R, WANG P, DEE L, SKORUPA G, KHADOUR F, SCHULZ R, PARRILLO JE: Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. *Am J Physiol* **276**: R265-R276, 1999.

KUMAR A, HAERY C, PARRILLO JE: Myocardial dysfunction in septic shock. *Crit Care Clin* **16**: 251-287, 2000.

KUMAR A, WOOD K, PARRILLO JE: Circulating substances and energy metabolism in septic shock. *Crit Care Med* **31**: 632-633, 2003.

LANCEL S, TISSIER S, MORDON S, MARECHAL X, DEPONTIEU F, SCHERPEREEL A, CHOPIN C, NEVIERE R: Peroxynitrite decomposition catalysts prevent myocardial dysfunction and inflammation in endotoxemic rats. *J Am Coll Cardiol* **43**: 2348-2358, 2004.

LANCEL S, JOULIN O, FAVORY R, GOOSSENS JF, KLUZA J, CHOPIN C, FORMSTECHER P, MARCHETTI P, NEVIERE R: Ventricular myocyte caspases are directly responsible for endotoxin-induced cardiac dysfunction. *Circulation* **111**: 2596-2604, 2005.

LEFER AM: Mechanisms of cardiodepression in endotoxin shock. *Circ Shock* **6** (Suppl 1): 1-8, 1979.

LEVY RJ, DEUTSCHMAN CS: Evaluating myocardial depression in sepsis. *Shock* **22**: 1-10, 2004.

MITAKA C, HIRATA Y, YOKOYAMA K, WAKIMOTO H, HIROKAWA M, NOSAKA T, IMAI T: Relationships of circulating nitrite/nitrate levels to severity and multiple organ dysfunction syndrome in systemic inflammatory response syndrome. *Shock* **19**: 305-309, 2003.

NATANSON C, EICHENHOLZ PW, DANNER RL, EICHACKER PQ, HOFFMAN WD, KUO GC, BANKS SM, MACVITTIE TJ, PARRILLO JE: Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. *J Exp Med* **169**: 823-832, 1989.

PARKER MM: Pathophysiology of cardiovascular dysfunction in septic shock. *New Horiz* **6**: 130-138, 1998.

PARRILLO JE, BURCH C, SHELHAMER JH, PARKER MM, NATANSON C, SCHUETTE W: A circulating myocardial depressant substance in humans with septic shock. Septic shock patients with a reduced ejection fraction have a circulating factor that depresses in vitro myocardial cell performance. *J Clin Invest* **76**: 1539-1553, 1985.

PARSONS PE: Interleukin-10: the ambiguity in sepsis continues. *Crit Care Med* **5**: 818-819, 1998.

PATHAN N, SANDIFORD C, HARDING SE, LEVIN M: Characterization of a myocardial depressant factor in meningococcal septicemia. *Crit Care Med* **3**0: 2191-2198, 2002.

PATHAN N, HEMINGWAY CA, ALIZADEH AA STEPHENS AC, BOLDRICK JC, ORAGUI EE, MCCABE C, WELCH SB, WHITNEY A, O'GARA P, NADEL S, RELMAN DA, HARDING SE, LEVIN M: Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock. *Lancet* **363**: 203-209, 2004.

REILLY JM, CUNNION RE, BURCH-WHITMAN C, PARKER MM, SHELHAMER JH, PARRILLO JE: A circulating myocardial depressant substance is associated with cardiac dysfunction and peripheral hypoperfusion (lactic acidemia) in patients with septic shock. *Chest* **95**: 1072-1080, 1989.

SOOP A, SOLLEVI A, WEITZBERG E, LUNDBERG JO, PALM J, ALBERT J: Exhaled NO and plasma cGMP increase after endotoxin infusion in healthy volunteers. *Eur Respir J* **21**: 594-599, 2003.

STRAND OA, LEONE A, GIERCKSKY KE, KIRKEBOEN KA: Nitric oxide indices in human septic shock. *Crit Care Med* **28**: 2779-2785, 2000.

YU X, CHEN Q, KENNEDY RH, LIU SJ: Inhibition of sarcoplasmic reticular function by chronic interleukin-6 exposure via iNOS in adult ventricular myocytes. *J Physiol Lond* **566**: 327-340, 2005a.

YU X, LIU MG, KENNEDY RH, CHEN Q: Both cGMP and peroxynitrite mediate chronic interleukin-6-induced negative inotropy in adult rat ventricular myocytes. *J Physiol Lond* **566**: 341-353, 2005b.

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