

Inhibition of p38 MAPK decreases myocardial TNF- α expression and improves myocardial function and survival in endotoxemia

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Abstract

Objectives: The role of p38 mitogen-activated protein kinase (MAPK) activation in lipopolysaccharide (LPS)-induced myocardial dysfunction has not been clearly defined. Our aim was to investigate the contribution of p38 MAPK in myocardial tumor necrosis factor- α (TNF- α) expression, cardiac function and survival during acute endotoxemia in mice. **Methods:** Acute endotoxemia was induced by LPS (10 mg/kg, i.p.) in mice. Two hours after LPS treatment, left ventricular (LV) function was assessed. Phosphorylation of p38 MAPK was measured by Western blotting. TNF- α mRNA and protein levels were determined by semi-quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. **Results:** LPS rapidly increased phosphorylation of p38 MAPK, followed by TNF- α mRNA expression and protein expression in the LV myocardium. Pre-treatment of the p38 MAPK inhibitor SB202190 (2 mg/kg, i.p.) decreased TNF- α mRNA and protein by 65 and 36%, respectively ($P < 0.05$). Immunohistochemical staining confirmed that cardiomyocytes were the major source of TNF- α production in the myocardium and blocking p38 MAPK activation inhibited TNF- α expression in response to LPS. Pre-treatment of SB202190 or a TNF- α antagonist etanercept (2 mg/kg, i.p.) significantly reversed LPS-induced LV depression ($P < 0.05$). LPS (20 mg/kg, i.p.) induced 94% mortality in mice within 72 h and pre-treatment with SB202190 and etanercept decreased LPS-induced mortality to 65 and 40%, respectively ($P < 0.01$). **Conclusion:** p38 MAPK activation represents an important mechanism leading to myocardial TNF- α production and cardiac dysfunction during acute endotoxemia in mice. Our data suggest that p38 MAPK is a potential therapeutic target of endotoxemia.

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Keywords: Cytokines; Endotoxins; Protein phosphorylation; Signal transduction; Ventricular function

1. Introduction

Sepsis is one of the main consequences of infectious diseases and occurs in 2–11% of all hospital or intensive care unit admissions. Mortality is 20–30% in sepsis and 40–80% in septic shock [1]. Myocardial dysfunction is a common complication of septic shock [2,3]. Endotoxins or lipopolysaccharides (LPSs) of Gram-negative bacteria are important pathogens responsible for myocardial depression. Studies from human volunteers and animal models

have shown that LPSs mimic the cardiac depression of septic shock [4,5]. LPS-stimulated production of proinflammatory cytokines, in particular tumor necrosis factor- α (TNF- α), has been proposed as one of the main mechanisms for cardiac dysfunction [5].

It has been demonstrated that cardiomyocytes synthesize TNF- α in response to LPS exposure [6,7]. TNF- α impairs contractile performance in intact animals [8], isolated hearts [7] and cardiomyocytes [9]. Furthermore, LPS-induced myocardial depression was completely abrogated in the presence of TNF- α antiserum [7] and administration of TNF binding proteins preserved myocardial function in endotoxemic rats [10]. These studies suggest that inhibi-

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tion of TNF- α bioactivity is of therapeutic potential for LPS-induced myocardial dysfunction. However, the cellular and molecular mechanism by which LPSs induce TNF- α expression in myocardium remains largely unknown.

The mammalian p38 mitogen-activated protein kinase (MAPK) was originally identified in murine pre-B lymphocytes transfected with the LPS-complex receptor CD14 and in murine macrophages where it was activated in response to LPS [11]. Since then, p38 MAPK has been found to influence a multitude of cellular events, such as cell growth and death, cell proliferation, differentiation and inflammation [12]. In the heart, p38 MAPK is activated in cardiac myocytes by cellular stress and hypertrophic stimuli, and is implicated in hypertrophy, survival and apoptosis of cardiomyocytes [13,14]. In response to LPS, activation of p38 MAPK has been demonstrated to be an essential signaling mechanism governing the regulation of TNF- α expression in the neutrophils and macrophages [15,16]. However, significant differences appear to exist between different cell types with respect to the role of p38 MAPK in TNF- α synthesis. For example, in the mast cell, production of TNF- α is not linked to activation of p38 MAPK [17,18]. These differences indicate that the role of p38 MAPK activation in TNF- α synthesis is likely cell type specific. In this regard, we have recently demonstrated that LPS rapidly increased phosphorylation of p38 MAPK in cultured neonatal mouse cardiomyocytes and inhibition of p38 MAPK activation abrogated LPS-induced TNF- α expression, suggesting an important role of p38 MAPK signaling in LPS-induced TNF- α production in cardiomyocytes *in vitro* [19]. However, the role of p38 MAPK in myocardial dysfunction during endotoxemia *in vivo* remains to be determined. The aim of the present study was to investigate the contribution of p38 MAPK to myocardial TNF- α expression, cardiac function and survival during acute endotoxemia in mice by pharmacological inhibition of p38 MAPK activation.

2. Methods

2.1. Animals

The investigation conforms with the *Guide for the Care and Use of Laboratory* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All experimental procedures were approved by the Animal Use Subcommittee at the University of Western Ontario, Canada. C57BL/6 mice were purchased from Jackson Laboratory. A breeding program was carried out at the London Health Science Center. Adult (3–4 months old) male mice weighing 21–26 g were studied.

2.2. Experimental protocol

Mice were randomly assigned to the following five

treatment groups: saline, SB202190, LPS, LPS plus SB202190 and LPS plus etanercept. Mice were treated intraperitoneally (i.p.) with a p38 MAPK inhibitor SB202190 (2 mg/kg) or saline, 30 min later, followed by LPS (10 mg/kg, $n=9$ mice/group). Seven mice were pretreated with etanercept (2 mg/kg, i.p.), a TNF- α blocking protein containing the extracellular ligand-binding portion of the p75 TNF- α receptor (recombinant human TNFR:Fc, Immunex, Seattle, WA, USA) [20] and LPS (10 mg/kg, i.p.) was given 1 h later. Mice treated with saline ($n=7$) or SB202190 alone ($n=5$) served as respective controls. Left ventricular (LV) function was assessed 2 h after LPS or saline treatment during anesthesia. At the end of hemodynamic measurement, mice were euthanized, and the LV myocardium and plasma were collected and stored at -70°C . For time course experiments, mice were treated with LPS (10 mg/kg, i.p.) and sacrificed at 15 min, 30 min, 1, 2 and 4 h ($n=5$ for each time point). The LV myocardium and plasma were collected for measurements of TNF- α expression and p38 MAPK phosphorylation.

Survival was monitored in the following three groups of mice: (1) Saline pretreatment followed by LPS (20 mg/kg, i.p., $n=17$); (2) SB202190 pretreatment (2 mg/kg, i.p.) followed by LPS (20 mg/kg, i.p., $n=17$); (3) etanercept pretreatment (2 mg/kg, i.p.) followed by LPS (20 mg/kg, i.p., $n=15$). LPS was administered 30 min after saline and SB202190 treatment, and 1 h after etanercept administration.

2.3. Hemodynamic measurements

Mice were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) and xylazine (15 mg/kg). Measurements of LV pressure were obtained using a tip-transducer catheter (1.4F, Millar Instruments, TX, USA) as we previously described [21]. Briefly, the catheter was calibrated at the beginning of each experiment. The Millar catheter was inserted into the right carotid artery for recording the arterial pressure under a dissecting microscope. The catheter was then advanced retrograde into the LV for recording of LV pressures. Pressure signals were fed to an analog digital converter and collected by a computer. Heart rate, LV systolic pressure, LV end-diastolic pressure (LVEDP), and maximal positive and minimal negative first derivative of LV pressure ($+dP/dt_{\text{max}}$ and $-dP/dt_{\text{min}}$) were analyzed by PowerLab software.

2.4. Analysis of TNF- α mRNA by reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from the LV myocardium using the Trizol Reagent (Gibco-BRL) following the manufacturer's instructions. Semi-quantitative RT-PCR for amplification of TNF- α in relation to glyceraldehyde-3-

phosphate dehydrogenase (GAPDH) was performed as we previously described [19].

2.5. Measurement of TNF- α protein

TNF- α protein levels in the LV myocardium and plasma were determined using a mouse TNF- α enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, USA) according to the manufacturer's instructions. The LV tissues were homogenized in phosphate-buffered saline (PBS). After centrifugation, the supernatant was collected for protein concentrations. TNF- α measurements were standardized with sample proteins and the plasma volume.

2.6. Immunohistochemistry

Sections of formalin-fixed and paraffin-embedded myocardial tissue were analyzed by an indirect immunoperoxidase technique [22]. Briefly, 5- μ m sections were incubated sequentially with primary antibody overnight at 4 °C, and then with peroxidase-conjugated donkey anti-goat IgG antibody. Each incubation was followed by three washes with PBS. The reaction was developed with diaminobenzidine and H₂O₂. The reaction was terminated by washing with PBS. Sections were counterstained with hematoxylin, dehydrated and mounted by routine methods. Goat polyclonal antibody (IgG) specific for TNF- α was purchased from Santa Cruz (USA).

2.7. Analysis of p38 MAPK phosphorylation

Assessment of the phosphorylation status of p38 MAPK in the LV myocardium was accomplished by Western blotting [19]. Briefly, aliquots containing 30 μ g of protein were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using 12% gels, followed by electrotransfer to ECL membranes. Blots were probed with antibodies against p38 MAPK/phospho-p38 MAPK (New England Biolabs, 1/1000), followed by incubation with horseradish peroxidase-conjugated secondary antibody (Bio-Rad). Detection was performed using an ECL chemiluminescence detection method. Signals were determined by densitometry. Levels of phosphorylated p38 MAPK to total p38 MAPK were presented.

2.8. Statistical analysis

All data were given as mean \pm S.D. Differences between two groups were compared by unpaired Student's *t*-test. For multigroup comparisons, analysis of variance (ANOVA) followed by Student–Newman–Keuls test was performed. Survival curves were created by the method of Kaplan and Meier, and compared by log-rank test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Phosphorylation of p38 MAPK and TNF- α expression in response to LPS in the myocardium

Consistent with what we showed in cultured neonatal mouse cardiomyocytes [19], LPS treatment in vivo induced an increase in phosphorylation of p38 MAPK in the adult mouse myocardium. Fig. 1 illustrates the time course of p38 MAPK phosphorylation in the LV myocardium after an intraperitoneal LPS administration. LPS rapidly increased phosphorylation of p38 MAPK and reached the maximum around 15–30 min and returned to basal levels after 4 h. TNF- α mRNA expression in response to LPS started within 15 min and reached the maximal level around 1 h in the LV myocardium (Fig. 2). The sequential activation of p38 MAPK and TNF- α expression after LPS treatment suggests a causal relationship between LPS-induced p38 MAPK activation and TNF- α expression in the mouse myocardium in vivo. In order to clarify that increased phosphorylation of p38 MAPK and induction of TNF- α expression in the myocardium did not result from the circulating TNF- α after LPS administration, we measured TNF- α levels in the plasma. After LPS treatment, TNF- α protein was not detected in the plasma at 15 min, but it was dramatically increased at 30 min and reached the

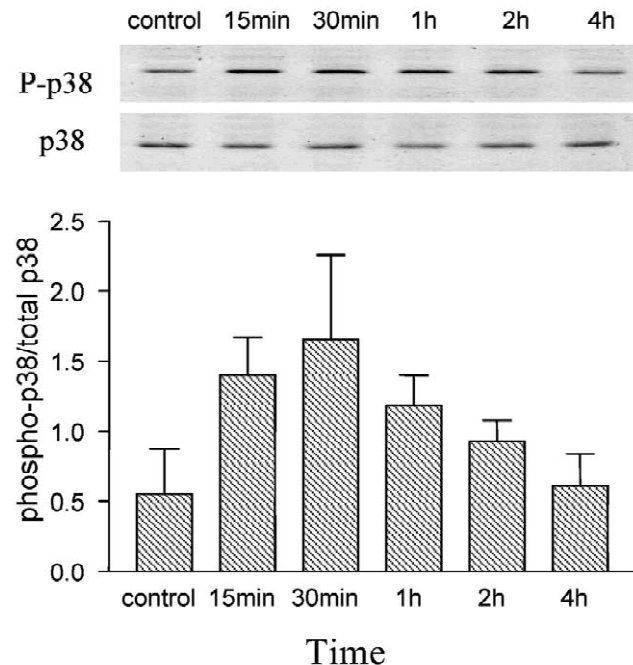


Fig. 1. Time course of p38 MAPK phosphorylation in the left ventricular (LV) myocardium in vivo. Mice were treated with LPS (10 mg/kg body weight, i.p.) for 15, 30 min, and 1, 2 and 4 h, respectively. LV tissues were collected and p38 MAPK phosphorylation was determined by Western blot using a specific antibody against phospho-p38 MAPK. The upper panel is a representative Western blot. The lower panel is the quantitative data showing LPS rapidly increased phosphorylation of p38 MAPK and reached the maximum around 15–30 min and returned to basal levels after 4 h. Data are mean \pm S.D. $n = 5$ per group.

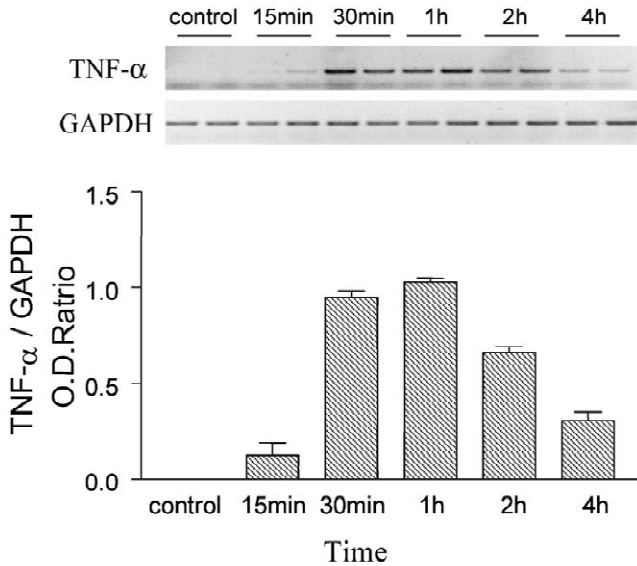


Fig. 2. Time course of TNF- α mRNA expression in the left ventricular (LV) myocardium in vivo. Mice were treated with LPS (10 mg/kg body weight, i.p.) for 15, 30 min, and 1, 2 and 4 h, respectively. Left ventricular tissues were collected and TNF- α mRNA expression was analyzed by semi-quantitative RT-PCR. The upper panel is a representative gel for the amplification of TNF- α mRNA products. The lower panel is the quantitative data from RT-PCR analysis. In response to LPS, TNF- α mRNA expression started within 15 min and reached the maximal level around 1 h in the LV myocardium in response to LPS. Data are mean \pm S.D. $n=5$ per group.

maximum at 2 h (Fig. 3). The result indicates that increased phosphorylation and induction of TNF- α in the myocardium preceded the appearance of circulating TNF- α in response to LPS. This excludes such possibility that increased phosphorylation of p38 MAPK and induction of

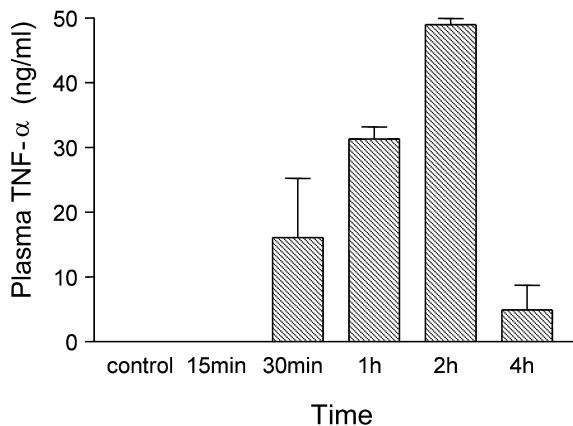


Fig. 3. Time course of TNF- α protein in plasma after LPS treatment. Mice were treated with LPS (10 mg/kg body weight, i.p.) for 15, 30 min, and 1, 2 and 4 h, respectively. Blood was collected and plasma was obtained by centrifugation. TNF- α protein was measured by ELISA. TNF- α protein was not detectable in plasma at 15 min, but it was dramatically increased at 30 min and reached the maximum at 2 h after LPS treatment. Data are mean \pm S.D. $n=5$ per group.

TNF- α expression in the myocardium were stimulated secondary to the circulating TNF- α induced by LPS administration.

3.2. Effect of p38 MAPK inhibitor on myocardial TNF- α expression stimulated by LPS

We have recently demonstrated that p38 MAPK activation is required for LPS-induced TNF- α expression in cultured neonatal mouse cardiomyocytes [19]. In order to determine whether this is also the case in the myocardium in vivo, the effect of p38 MAPK inhibition on LPS-induced TNF- α expression was assessed in the whole animal model. LPS treatment for 2 h significantly increased TNF- α protein levels in the myocardium compared to sham ($P<0.01$). Pre-treatment of a selective p38 MAPK inhibitor, SB202190 (2 mg/kg, i.p.) for 30 min significantly decreased TNF- α mRNA ($P<0.05$, $n=5$) and protein expression ($P<0.05$, $n=5$) in the adult LV myocardium (Fig. 4A and B). SB202190 alone did not affect TNF- α protein levels in the myocardium. These results demonstrated a causal relationship between p38 MAPK activation and TNF- α expression in the adult myocardium in vivo during LPS stimulation. Since LPS increased TNF- α levels in plasma as demonstrated in Fig. 3, we also investigated the effect of p38 MAPK inhibitor on TNF- α in plasma 2 h after LPS stimulation. Plasma levels of TNF- α were not detectable in saline and SB202190 groups (Fig. 5). Pre-treatment of SB202190 (2 mg/kg) for 30 min significantly decreased LPS-induced TNF- α protein levels ($P<0.05$, $n=5$) in plasma (Fig. 5). These data suggest p38 MAPK activation also played an important role in modulating the circulating TNF- α stimulated by LPS in mice.

3.3. Localization of TNF- α expression in the myocardium

In order to localize the TNF- α expression in the myocardium, immunohistological staining of myocardial tissues was carried out using a TNF- α specific antibody. As shown in Fig. 6, no positive signal was found in the myocardium from the sham animal (Fig. 6A). Upon LPS stimulation for 2 h, almost all of the cardiomyocytes were stained positive for TNF- α in the myocardium. The positive signals were localized in the cytoplasm of cardiomyocytes (Fig. 6B). Pre-treatment with SB202190 for 30 min decreased TNF- α staining in the myocardium induced by LPS (Fig. 6C). The results further confirmed that cardiomyocytes were the major source of TNF- α production in the myocardium and blocking p38 MAPK activation inhibited TNF- α expression in response to LPS.

3.4. Hemodynamic changes

In response to LPS (10 mg/kg, i.p.), mean arterial

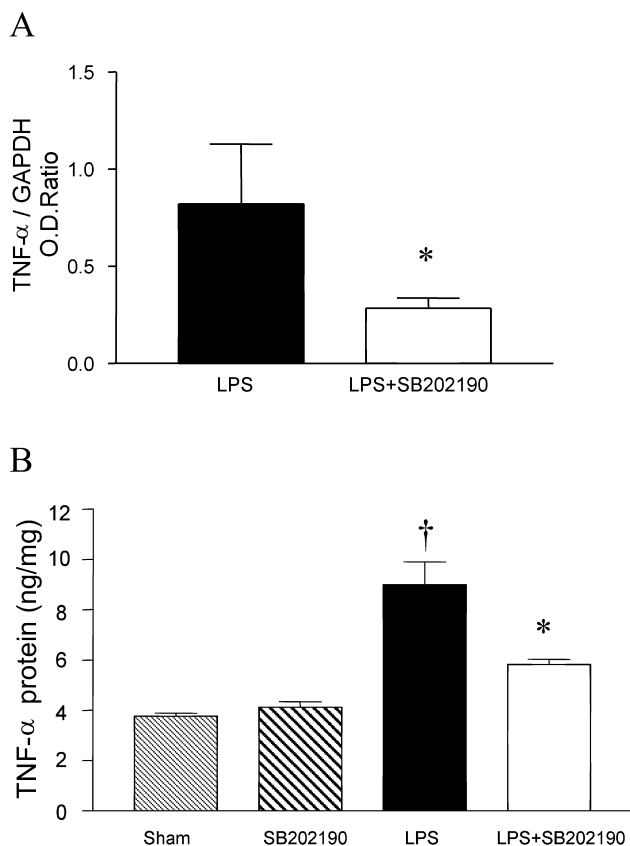


Fig. 4. Inhibition of p38 MAPK activation decreased TNF- α expression stimulated by LPS in the left ventricular (LV) myocardium in vivo. Mice were treated intraperitoneally with SB202190 (2 mg/kg body weight) or saline for 30 min, followed by LPS (10 mg/kg body weight) for 2 h. The LV myocardium was collected. (A) TNF- α mRNA expression by semi-quantitative RT-PCR. Pre-treatment with SB202190 decreased TNF- α mRNA expression in response to LPS. (B) Myocardial TNF- α protein production. LPS significantly increased myocardial TNF- α protein production compared to sham and SB202190 groups ($\dagger P < 0.01$). Pre-treatment with SB202190 significantly decreased LPS-induced TNF- α protein production in the LV myocardium ($* P < 0.05$). Data are mean \pm S.D. $n = 5$ per group.

pressure (MAP) was significantly decreased, whereas heart rate was significantly increased ($P < 0.05$, Table 1). Left ventricular systolic pressure (LVSP), the maximal positive and minimal negative first derivatives of LV pressure ($LV + dP/dt_{max}$ and $-dP/dt_{min}$) were significantly reduced in endotoxemic mice compared with sham animals ($P < 0.05$, Table 1), indicating myocardial depression. SB202190 alone did not have any effects on any of the parameters. Pretreatment of SB202190 (2 mg/kg, i.p.) induced a significant increase of $LV + dP/dt_{max}$ and $-dP/dt_{min}$ ($P < 0.05$) without affecting MAP, HR or LVSP in endotoxemic mice. Furthermore, pretreatment with etanercept (2 mg/kg, i.p.) significantly increased LVSP, $LV + dP/dt_{max}$ and $-dP/dt_{min}$ in LPS-treated mice (Table 1, $P < 0.05$). These results demonstrated that inhibition of p38 MAPK and TNF- α ameliorated cardiac dysfunction induced by LPS in mice.

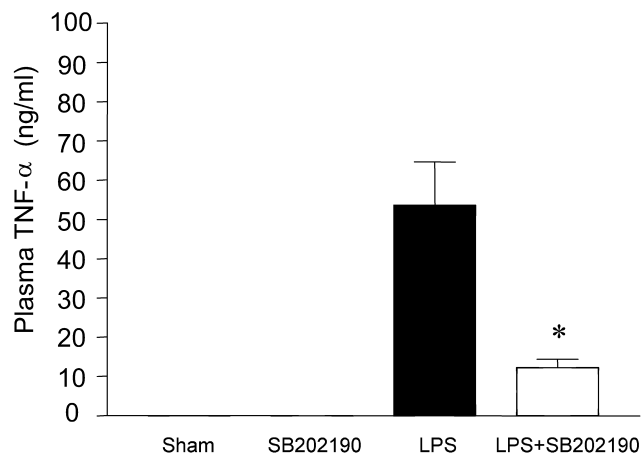


Fig. 5. Reduction of LPS-induced TNF- α production in plasma by inhibition of p38 MAPK. The plasma was collected 2 h after saline, SB202190 or LPS treatment and TNF- α concentrations were determined by ELISA. Plasma TNF- α levels in sham and SB202190 groups were undetectable. Pre-treatment with SB202190 for 30 min significantly decreased TNF- α production in plasma in response to LPS ($* P < 0.05$). TNF- α levels were expressed as ng/ml plasma. Data are mean \pm S.D. $n = 5$ per group.

3.5. Survival

LPS-treated (20 mg/kg, i.p.) mice showed signs of sepsis such as apathy, fur ruffling, conjunctivitis, and diarrhea. Fig. 7 shows the survival curves for SB202190- (2 mg/kg, i.p.), etanercept- (2 mg/kg, i.p.) and saline-pretreated mice followed by LPS. At 24 h after LPS administration, 47% of saline-pretreated mice died, while 18% and no deaths occurred in SB202190-pretreated mice and etanercept-pretreated mice, respectively. By 72 h, 94% of saline-pretreated mice died. In contrast, only 65% of SB202190-pretreated mice and 40% of etanercept-pretreated mice died at 72 h. Therefore, pretreatment with p38 MAPK inhibitor and TNF- α antagonist significantly improved survival during acute endotoxemia in mice ($P < 0.01$).

4. Discussion

In the present study, we demonstrated that LPS resulted in an immediate and transient increase in p38 MAPK activation, which was followed by TNF- α production in the myocardium. Inhibition of p38 MAPK activation decreased myocardial TNF- α expression and improved cardiac function and survival during endotoxemia in mice. Our data support the notion that activation of p38 MAPK plays an important role in TNF- α expression in the myocardium and myocardial depression in endotoxemia.

Myocardial dysfunction is a common clinical manifestation in sepsis and septic shock [23]. Although the exact cause of myocardial depression during sepsis and

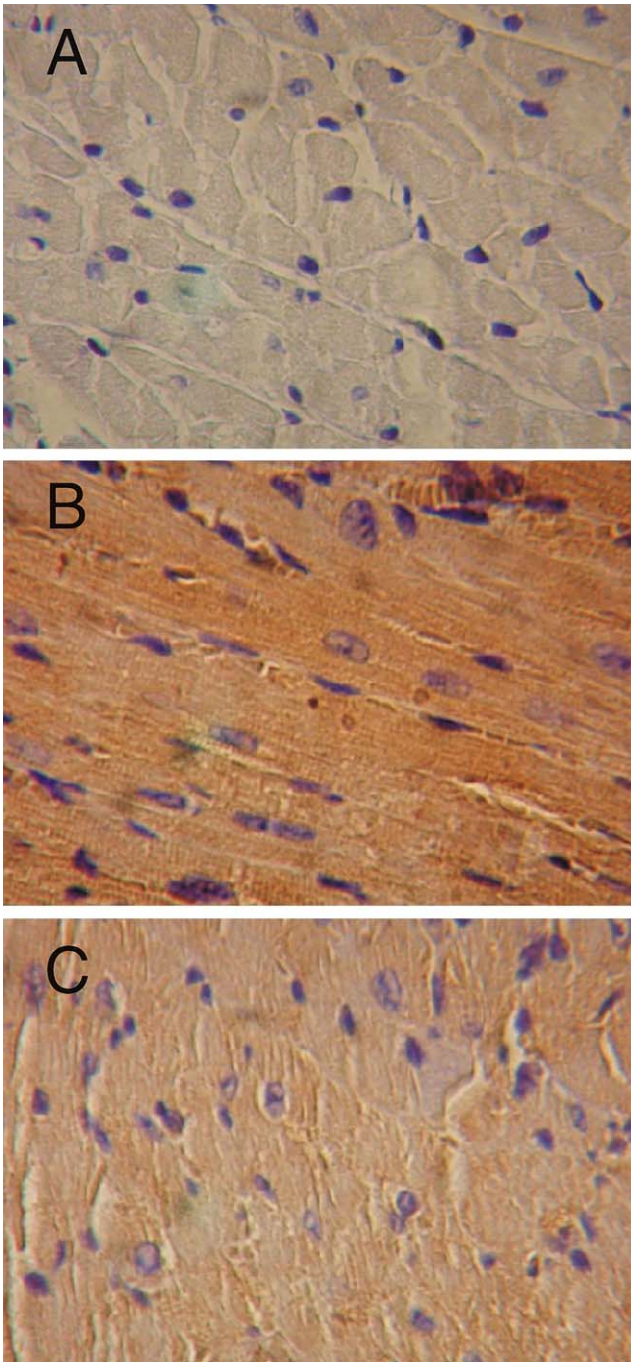


Fig. 6. Photomicrographs of TNF- α protein staining in the LV myocardium. Tissue sections from saline or LPS alone or pre-treatment with SB202190 followed by LPS for 2 h were processed for detection of TNF- α protein expression and its cellular location in the LV myocardium. (A) Saline treatment. No TNF- α staining was found in the myocardium from saline-treated mice. (B) LPS treatment. TNF- α staining was present in most cardiomyocytes and localized in the cytoplasm but not in the nuclei of cardiomyocytes. (C) SB202190 plus LPS treatment. Pretreatment with SB202190 decreased TNF- α staining in the cardiomyocytes induced by LPS. Sections are representatives of five mice from each group. (Magnification: 200 \times).

septic shock is not fully understood, production of cytokines such as TNF- α and interleukin-1 β is certainly involved [24]. Whereas macrophages and monocytes are considered to be a predominant source of circulating TNF- α in response to LPS stimulation [25], it is now clear that cardiomyocytes are the major local source of TNF- α in the myocardium [6–8]. However, the mechanism by which LPS stimulates TNF- α expression in cardiomyocytes has not been fully defined. We recently demonstrated that activation of p38 MAPK plays an important role in LPS-induced TNF- α expression in cultured neonatal mouse cardiomyocytes [19]. In the present study, treatment with LPS increased p38 MAPK phosphorylation which preceded myocardial TNF- α expression. Pre-treatment with SB202190, a selective inhibitor of p38 MAPK, markedly inhibited both TNF- α mRNA and protein expression in the myocardium induced by LPS. Furthermore, immunohistological staining showed that TNF- α expression was localized in the cytoplasm of cardiomyocytes and SB202190 administration decreased TNF- α staining signals in the myocardium from LPS-treated mice. Our data clearly indicate that p38 MAPK signaling is an important pathway leading to TNF- α expression in response to LPS stimulation in the myocardium *in vivo*.

Accumulating evidence suggest that p38 MAPK plays an important role in myocardial dysfunction in various animal models. For example, in a mouse model of ischemia and reperfusion, p38 MAPK was activated in the heart and disruption of a single copy of the p38 α MAPK gene decreased infarct size [26]. In a rat model of cardiac hypertrophy and dysfunction, hypertensive end-organ damage and premature mortality were shown to be p38 MAPK dependent [27]. Furthermore, transgenic mice with targeted activation of p38 MAPK in ventricular cardiomyocytes showed both systolic contractile depression and impaired diastolic function of the heart [28]. However, it remains unknown whether p38 MAPK has any effect on myocardial dysfunction during sepsis. In the present study, we demonstrated for the first time that LPS treatment resulted in a marked activation of p38 MAPK in the myocardium and significant decreases in both systolic and diastolic function. Furthermore, treatment with SB202190, a highly specific p38 MAPK inhibitor, ameliorated myocardial dysfunction and improved survival. These results suggest that activation of p38 MAPK contributes to myocardial dysfunction and high mortality during endotoxemia in mice.

Mechanisms by which p38 MAPK activation leads to myocardial dysfunction are not fully understood. A recent study have demonstrated that p38 MAPK activation leads to myocardial TNF- α production and cardiac dysfunction during burn trauma [29]. Studies have shown that TNF- α expression contributes significantly to myocardial dysfunction in endotoxemia [7,10]. In the present study we demonstrated that inhibition of p38 MAPK activation decreased myocardial TNF- α expression and improved

Table 1

Effects of p38 MAPK inhibitor SB202190 and TNF- α antagonist etanercept on hemodynamics in mice treated with LPS

Parameter	Sham	SB202190	LPS	LPS+SB202190	LPS+etanercept
<i>n</i>	7	5	8	9	6
MAP, mmHg	99.8 \pm 14.8	87.3 \pm 2.8	56.9 \pm 5.1*	66.1 \pm 13.5	75.8 \pm 2.4†
HR, beats/min	407.2 \pm 41.5	378.5 \pm 8.8	447.9 \pm 30.7	461.0 \pm 28.2	442.6 \pm 9.4
LVEDP, mmHg	3.2 \pm 1.3	4.9 \pm 0.8	3.4 \pm 1.8	2.5 \pm 1.6	3.1 \pm 0.5
LVSP, mmHg	117.6 \pm 17.4	115.1 \pm 5.4	81.4 \pm 4.5*	88.2 \pm 11.5	106.7 \pm 2.3†
+dP/dt _{max} , mmHg	9269.3 \pm 1411.5	8908.1 \pm 435.3	4351.8 \pm 723.7*	5495.1 \pm 1342.2†	8036.5 \pm 155.08†
-dP/dt _{min} , mmHg	9240.3 \pm 1297.7	8693.2 \pm 296.9	5249.1 \pm 878.8*	6246.5 \pm 1005.8†	8770.0 \pm 140.3†

MAP, Mean arterial pressure; HR, heart rate; LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; LV +dP/dt_{max} and -dP/dt_{min}, maximal positive and minimal negative first derivative of LV pressure. All data are mean \pm S.D. * P <0.05 compared with Sham; † P <0.05 compared with LPS-treated mice.

myocardial function during endotoxemia. In order to demonstrate the specific role of TNF- α in endotoxemia, etanercept—a fusion protein that blocks TNF- α was utilized. Etanercept also improved cardiac function and survival in endotoxemic mice. Our results suggest that attenuation of TNF- α expression represents an important mechanism by which inhibition of p38 MAPK improves myocardial function and survival in endotoxemia. Activation of p38 MAPK has also been shown to induce myocyte apoptosis and increase the expression of adhesion molecules during myocardial ischemia and reperfusion [30,31], and decrease myofilament response to Ca²⁺ in cultured adult rat cardiomyocytes [32]. Whether these effects of p38 MAPK contribute to myocardial dysfunction in endotoxemia require further investigation. In the present study, inhibition of TNF- α by SB202190 or etanercept increased LV contractility and survival suggests the contribution from improvement of cardiac function. It is possible that the extra-cardiac effects of SB202190 and etanercept may also

contribute to the improved survival in the endotoxemic mice. The nature of these effects and their relationship to the cardiac effects of SB202190 and etanercept remain to be investigated.

In summary, we demonstrated that LPS treatment resulted in significant activation of p38 MAPK in the myocardium. We showed for the first time that activation of p38 MAPK plays an important role in the signal pathway leading to myocardial TNF- α expression in response to LPS in vivo. Furthermore, inhibition of p38 MAPK and TNF- α significantly improved myocardial function and survival during endotoxemia. Thus, p38 MAPK activation represents an important mechanism leading to myocardial TNF- α production and cardiac dysfunction during acute endotoxemia. Our study suggests that p38 MAPK is a therapeutic target of endotoxemia.

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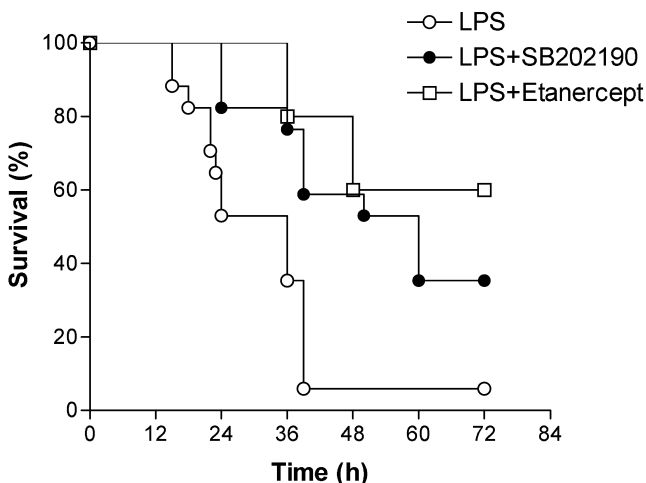


Fig. 7. Kaplan and Meier survival curves of endotoxemic mice. Mice were pretreated with saline, SB202190 (2 mg/kg body weight, i.p.) or etanercept (2 mg/kg body weight, i.p.) followed by LPS (20 mg/kg body weight, i.p.) as described in Methods. After LPS administration, survival of mice was monitored at every 12 h for 72 h. Survival was significantly increased in LPS+SB202190 ($n=17$) and LPS+etanercept ($n=15$) groups compared to LPS group ($n=17$, P <0.01).

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