Our current understanding of the pathogenesis of sepsis suggests that bacteria as well as bacterial-derived products activate an uncontrolled network of host-derived mediators such as proinflammatory cytokines (e.g., tumor necrosis factor [TNF] and interleukin [IL]-1β), which can ultimately lead to cardiovascular collapse and death. Despite the potentially important role that TNF and IL-1β may play in producing cardiac dysfunction in human septic shock, little is known with regard to the basic biochemical mechanism(s) by which bacterial pathogens induce their expression in the heart. A major advance in understanding the early events that are downstream from bacterial-mediated signaling has been the identification of Toll-like receptors (TLRs). TLR-mediated signaling is known to activate the transcription factor nuclear factor-κB and to upregulate TNF expression. It has recently been shown that the heart expresses TLRs, raising the possibility that these receptors may be responsible for mediating the deleterious effects of bacterial pathogens on cardiac function. In this review, we will discuss the emerging role for TLRs in the pathogenesis of the cardiovascular collapse that occurs during sepsis.

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**Key words:** cytokines; innate immunity; myocardial dysfunction; septic shock; Toll-like receptors

**Abbreviations:** IL = interleukin; LPS = lipopolysaccharide; NF = nuclear factor; NO = nitric oxide; PAMP = pathogen-associated molecular pattern; PRR = pattern recognition receptor; TLR = Toll-like receptor; TNF = tumor necrosis factor

In critically ill patients, sepsis has a mortality rate of 30 to 50%, and it is the 13th leading reversible cause of death in the United States. Septic patients frequently do not die from their primary infection but from progressive multiple-organ failure. In the initial response to a localized infection, the release of endotoxins or exotoxins by a bacterial infection induces tissue macrophages (as well as other cells) to generate inflammatory cytokines, including tumor necrosis factor (TNF) and interleukin (IL)-1β. Although these early-response cytokines play an important part in host defense by attracting activated neutrophils to the site of infection, the entry of these cytokines and bacterial products into the systemic circulation can bring about widespread microvascular injury. Thus, activation of the innate immune response following systemic infection may be regarded as a mixed blessing. There is a clear-cut benefit of eliminating and/or containing microorganisms at the site of entry into the host; however, there are deleterious end-organ effects, such as myocardial depression, that can be attributed to the excessive production of proinflammatory mediators.
TNF, IL-1β, and nitric oxide (NO) have been implicated in a number of in vivo and ex vivo studies\(^4,7\) as important mediators of myocardial dysfunction. We and others\(^3,5,7\) have documented an increase in myocardial TNF after lipopolysaccharide (LPS) stimulation, partly synthesized locally by the cardiac myocytes themselves. Given that a reduction in ejection fraction occurs in 40% of septic patients, local myocardial TNF levels may be an important factor in the progression of myocardial dysfunction during sepsis. Until recently, the molecular mechanisms by which LPS induces cytokine production in the heart had remained unknown. However, recent studies\(^6,7\) have documented that the heart itself possesses a functionally intact innate immune system and that this can be nonspecifically activated in response to various forms of injury. The purpose of this article is to review the molecular mechanism involved in the pathogenesis of LPS-induced cardiac inflammation and dysfunction focusing particular attention on the roles of Toll-like receptors (TLRs).

Epidemiology of Sepsis

Human sepsis is responsible for approximately 70,000 deaths per year in the United States.\(^8\) Although the overall incidence of sepsis is unknown, it is estimated that about 400,000 to 500,000 cases occur each year in this country.\(^1,9,10\) Based on discharge diagnosis codes for bacteremia and septicemia, the Centers for Disease Control and Prevention recently reported a 139% increase in the incidence of sepsis over a 10-year period.\(^1\) Although great improvements have been made in the supportive care of critically ill patients,\(^11,12\) the mortality rate has remained high when an acute infection induces sepsis with shock, metabolic acidosis, oliguria, or hypoxemia. In the United States, the mortality rate ranges from 30 to 50%, even with intensive medical care, including antibiotics, IV fluids, nutrition, mechanical ventilation for respiratory failure, and surgery when indicated to eradicate the source of infection.\(^13\)

A large number of endogenous plasma factors, cells, and cell products have been implicated in the pathogenesis of sepsis. However, TNF is the cytokine that has most often been associated with sepsis/septic shock for a number of reasons. First, TNF plasma levels are elevated during experimental and clinical sepsis\(^14,15\); second, increasing concentrations of TNF during sepsis are associated with increasing mortality\(^14\); third, exogenous TNF administration in humans and animals leads to/or simulates sepsis and organ failure; and fourth, at least in animal models, TNF neutralization frequently leads to amelioration of sepsis symptoms and improves survival.\(^16,17\) Based on these observations and in an effort to improve survival in humans, adjunctive therapies directed against TNF were produced and a number of clinical trials were conducted. Unfortunately, studies\(^18–23\) using monoclonal antibodies directed against TNF or soluble TNF receptors failed to significantly improve survival in septic patients. Thus, it became clear that a better understanding of the molecular mechanisms leading to the induction of the innate immune response during sepsis was essential in developing adjunctive therapies that could improve both morbidity and mortality.

Innate Immunity and Sepsis: the Role of Pattern Recognition Receptors

The immune system has traditionally been divided into innate and adaptive components, each of which has a different role and function in defending the host against infectious agents. The innate immune response is a preprogrammed nonspecific first line of defense that is primarily responsible for eliminating and/or containing pathogens at the site of entrance into the host. To impede the entrance of infectious microorganisms, the innate immune system has developed a series of phylogenetically conserved receptors, termed pattern recognition receptors (PRRs), that have the ability to recognize specific pathogen-associated molecular patterns (PAMPs), allowing the innate immune system to distinguish self molecules from the pathogen-associated nonself structures.\(^24\)

Examples of PAMPs include LPS of Gram-negative organisms, the teichoic acids of Gram-positive organisms, and the glycolipids of mycobacteria. Recognition of PAMPs by PRRs results in the activation of different intracellular cascades, which in turn lead to the expression of effector molecules such as cytokines, chemokines, and adhesion molecules that are involved in inflammation. Until recently, this evolutionary conserved system was primarily described in cells of the immune system, but it is now known to exist in various tissues where its activation may play a significant role in the pathogenesis of sepsis-induced multiorgan dysfunction.

Specific members of the innate immune system include PRRs such as CD14, and the newly described TLRs. CD14 is a 55-kd glycosylphosphatidylinositol-anchored receptor that binds LPS with high affinity and is critically involved in mediating LPS responses. The most compelling evidence for the importance of CD14 as a LPS-associated signal transducer came from genetic studies. Ferrero et al\(^25\) showed that mice overexpressing CD14 were hypersensitive to LPS. Subsequently, CD14-deficient mice were shown to be protected against LPS-induced septic shock and, in fact, were 10,000-fold
less sensitive to LPS than control littermates. However, given that CD14 lacks a transmembrane domain, the mechanism by which LPS binding to CD14 induces cell activation leading to proinflammatory cytokine production had remained an enigma.

One of the major advances in the understanding of early events in microbial recognition and subsequent development of sepsis has been the identification of TLRs. Medzhitov et al described the first human homolog of Drosophila Toll, termed human TLR4. TLR4 was shown to activate nuclear factor (NF)-κB and induce the expression of a number of proinflammatory cytokines in response to LPS. Soon afterwards, additional human TLRs were discovered; to date, nine TLRs have been extensively characterized. Poltorak et al and Qureshi et al were the first to demonstrate that the genetic defect in two strains of mice that are hyporesponsive or nonresponsive to LPS is linked to TLR4. The gene locus responsible for this defect (Ipsd) was mapped to the Tlr4 gene. In the C3H/HeJ mouse, a single missense mutation within the Tlr4 coding sequence was identified. C3H/HeJ mice are homozygous for a mutation that substitutes histidine for proline at position 712. In contrast, C57Bl/10ScCr and C57Bl/10ScN strains were found to be null for the Tlr4 gene. Evidence further supporting the hypothesis that TLR4 is the LPS receptor comes from the finding that a targeted disruption of the Tlr4 gene also renders normal mice resistant to LPS. These studies provided a direct link between TLR4 and physiologic responses to LPS. More importantly, they provided critical information about the membrane receptor for LPS by providing a link between LPS binding to CD14 and transmembrane signaling via TLR4.

Although TLR2 was initially linked to LPS signaling, genetic evidence now supports that TLR4 is the primary signal transducer for LPS. Studies in TLR2-deficient mice have confirmed the minimal role of this receptor in LPS signaling; these mice are susceptible to the toxic effect of LPS. However, studies indicate that TLR2, but not TLR4, is the receptor for Gram-positive organisms (i.e., Staphylococcus aureus and Streptococcus pneumoniae) and their cell wall components, as well as for bacterial lipoproteins and yeast.

**TLR Signal Transduction Pathways**

Although TLR proteins can recognize both Gram-negative and Gram-positive bacteria, LPS has been used most often as a stimulus when examining the signal transduction pathway for both TLR2 and TLR4. The engagement of TLRs by microbial products leads to the activation of multiple intracellular signal transduction pathways. Among the best-characterized pathways is the one leading to NF-κB activation (Fig 1). TLRs most likely form homodimers, leading to a conformational change in the cytoplasmic Toll/IL-1R module with subsequent recruitment of an adapter protein named MyD88. MyD88 consists of a C-terminal domain that binds TLR via the cytoplasmic Toll/IL-1R module and an N-terminal portion that is a death-domain module. The death-domain module of MyD88 recruits IL-1 receptor-associated kinase to the receptor complex. IL-1 receptor-associated kinase is then autophosphorylated and dissociates from the receptor complex and recruits TNF receptor-associated factor 6, which in turn activates downstream kinases. NF-κB–inducing kinase finally activates the inhibitory κB kinase complex that directly phosphorylates IκBα leading to nuclear translocation of NF-κB and initiation of gene transcription. The dysregulated activation of NF-κB by bacteria or bacterial products may lead to the excessive production of proinflammatory mediators, resulting in tissue damage, organ failure, and even death to the host as can be seen in overwhelming bacterial sepsis.

**Myocardial Depression in Sepsis: Role of Proinflammatory Mediators**

Since septic shock is the most common early manifestation of severe sepsis, the effects of sepsis...
on myocardial function have been of clinical interest for many years. The classic pattern of cardiac function in septic shock includes the following group of findings: (1) a reduced left and right ventricular ejection fraction, (2) increased end-diastolic and end-systolic volumes for both right and left ventricles with preservation of stroke volume, (3) an elevated heart rate and cardiac output, and (4) a decrease in systemic vascular resistance. In the clinical setting, the reduction in ejection fraction and the biventricular dilatation are thought to occur within 24 after the onset of sepsis. However, animal models of endotoxic shock have suggested that the onset of myocardial dysfunction may occur as early as 2 h after LPS challenge. Experimental studies suggested a possible link between bacterial species and cardiac dysfunction. However, Jafri et al reported that echocardiographic parameters (such as left ventricular end-diastolic volume, left ventricular end-systolic volume, and left ventricular ejection fraction) recorded in patients with Gram-positive or Gram-negative sepsis did not differ significantly. Thus, from the limited available data the severity of cardiac dysfunction associated with sepsis seems to be independent of the causative organism.

A circulating myocardial depressant factor in patients with septic shock was proposed 50 years ago, but it was not until the late 1980s that myocardial dysfunction was quantitatively linked to a serum factor. Although the precise endogenous agents that cause the myocardial depression in sepsis remain elusive, recent studies have identified the combination of TNF and IL-1β as playing an important role in the pathogenesis of sepsis-induced cardiac depression. Studies by Heard et al and Pagani et al also demonstrated that TNF was capable of eliciting the pattern of hemodynamic abnormalities and myocardial dysfunction observed in human septic shock. Linking shock to myocardial dysfunction, Kumar et al convincingly demonstrated that the myocardial depressant activity of human sera obtained from patients with septic shock could be eliminated by the immunoprecipitation of TNF and IL-1β. Cain et al subsequently demonstrated that TNF and IL-1β synergistically depress myocardial function in vitro and that an inhibitor of NO could abolish the effect of these two cytokines. More recently Ullrich et al demonstrated a role for NO in LPS-induced murine myocardial dysfunction in vivo. In this study, mice deficient in NO synthase 2 had normal left ventricular contractile function following challenge with LPS. However, other studies have found no evidence for a contribution of NO to the negative inotropic effects of TNF or LPS on the heart. Interestingly, Grandel et al demonstrated that the LPS-induced early loss in contractile func-

![Figure 2](image-url)
tion proceeds largely through TNF-induced sphingosine production and not through cardiac NO synthesis. Despite these elegant studies, the mechanisms by which these innate immune mediators are induced within the heart by bacteria or bacterial cell wall products remain to be defined.

Myocardial TLRs and Possible Link to Myocardial Inflammation and Dysfunction

Recent studies indicate that the adult mammalian myocardium possesses a functionally intact innate immune system. First, there is now substantial evidence that the mediators and effectors of the innate immune response including proinflammatory cytokines, NO, and chemokines are expressed in the adult mammalian heart by cardiac myocytes in response to challenge with classical PAMPs, such as LPS and viral particles. Second, the heart expresses at least four PRRs for PAMPs. For example, CD14, a PRR for LPS, is expressed by adult cardiac myocytes. Moreover, studies have identified the presence of human TLRs, including TLR2, TLR4, and TLR6, in the heart. Thus, a dysregulated innate immune response within the cardiac compartment may significantly contribute to the pathophysiology of sepsis-induced myocardial dysfunction.

Recently, we investigated the effect of a point mutation in TLR4 on cardiac inflammation in a model of endotoxic shock. LPS challenge induced a rapid and robust increase in TNF and IL-1β myocardial messenger RNA expression in control mice. In contrast, the LPS response in C3H/HeJ (TLR4-deficient) mice was significantly blunted and delayed (Fig 2, left, A and B). Intramyocardial TNF and IL-1β protein levels after LPS challenge were significantly higher in control mice (Fig 2, right, C and D). Similarly, myocardial NO synthase 2 activity and NO production were significantly greater in control mice. Activation of myocardial NF-κB was observed within 30 min of LPS challenge in control mice, but not in TLR4-deficient mice (Fig 3). These findings indicate that signaling via TLR4 is responsible, at least in part, for the induction of proinflammatory mediators in the heart during endotoxic shock.

Nemoto et al examined whether TLR4 was critical to the development LPS-induced myocardial dysfunction in vivo. Following LPS injection, TLR4-deficient mice and control mice underwent comparative echocardiograms before and 6 h after LPS administration. LPS challenge resulted in a significant depression of ejection phase indexes of contractile function (percent of fractional shortening and

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/ on 04/09/2017)

**Figure 3.** Myocardial NF-κB activation is impaired in LPS-challenged TLR4-deficient (TLR4-D; C3H/HeJ) mice. Crude nuclear extracts were prepared from myocardial tissue isolated from wild-type (WT) [C3HeB/FeJ] and TLR4-D mice at the indicated times after LPS challenge (25 mg/kg). Supershift experiments and cold chase (CC) assays were performed on samples collected 1 h after LPS challenge. From Baumgarten et al. Reproduced with permission from the University of Chicago Press (© 2001 by the Infectious Diseases Society of America; all rights reserved).
velocity of circumferential fiber shortening) in wild-type mice, whereas no significant changes in these parameters were noted in TLR4-deficient mice. This observation suggests that TLR4 activates downstream signaling pathways that are responsible for mediating LPS-induced left ventricular dysfunction. Whether CD14 and/or TLR2 are also critical in mediating LPS-induced left ventricular dysfunction. The exciting clinical implications of the discovery that TLR proteins recognize bacteria and/or bacterial cell wall components and that they are expressed in the heart are twofold. First, TLRs may be useful targets for the development of adjunctive therapies for the treatment of septic shock. Second, genetic studies may determine whether mutations in human TLR genes increase susceptibility to infection and progression to end-organ dysfunction during sepsis. By elucidating the function of TLRs and the mechanisms by which they activate cells, crucial information will be obtained to better understand how the host is able to mount an appropriate or inappropriate immune response. Considering that microorganisms such as Chlamydia may play a role in the pathogenesis of some forms of heart disease, the knowledge gained from studies of LPS-induced myocardial inflammation may also lead to novel therapeutic strategies for other cardiac diseases.

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REFERENCES

7 Haudek SB, Bryant DD, Girio BP. Differential regulation of myocardial NF-κB following acute or chronic TNF-α exposure. J Mol Cell Cardiol 2001; 33:1263–1271

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54 Klabunde RE, Coston AF. Nitric oxide synthase inhibition does not prevent cardiac depression in endotoxic shock. Shock 1995; 3:73–78


60 Frantz S, Kobzik L, Kim YD, et al. Tlr4 (TLR4) expression

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Downloaded From: http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/ on 04/09/2017
64 Frantz S, Kelly RA, Bourcier T. Role of TLR-2 in the activation of nuclear factor κB by oxidative stress in cardiac myocytes. J Biol Chem 2001; 276:5197-5203

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