REVIEW

Role of Toll-like receptors in cardiovascular diseases

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ABSTRACT

The discovery and characterization of the TLR (Toll-like receptor) family has led to a better understanding of the innate immune system. The strategy of innate immune recognition is based on the detection of constitutive and conserved products of micro-organisms. However, host molecules that are released during injury can also activate TLRs. Engagement of TLRs by microbial or host-derived molecules induces the expression of pro-inflammatory cytokines, which may have both beneficial and detrimental effects on the host. In addition to being expressed in immune cells, TLRs are expressed in other tissues such as those of the cardiovascular system. In the present review, the role of TLRs in septic cardiomyopathy, viral myocarditis, atherosclerosis, ischaemia/reperfusion injury and cardiac remodelling after myocardial infarction are outlined, with attention paid to genetically modified murine models. Although much has been learned about stress-induced TLR activation in the tissues of the cardiovascular system, the role of individual TLRs in initiating and integrating homeostatic responses within the heart remains to be defined. Accumulating evidence indicates that TLRs may play an important role in the pathogenesis of atherosclerosis, viral myocarditis, dilated cardiomyopathy, cardiac allograft rejection and sepsis-induced left ventricular dysfunction. Moreover, heart failure of diverse aetiology is also now recognized to have an important immune component, with TLR signalling influencing the process of cardiac remodelling and prognosis. In the present review, we outline the biology of TLRs as well as the current experimental and clinical evidence for the role of TLRs in cardiovascular diseases.

OVERVIEW OF TLRs (TOLL-LIKE 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 organism against infectious agents. The classic innate immune response is a preprogrammed, non-specific first line of defence that is primarily responsible for eliminating and/or containing micro-organisms at the site of encounter into the host. The strategy of innate immune recognition is based on the detection...
of constitutive and conserved products of microorganisms. Because the targets of innate immune recognition are conserved molecular patterns, they are called PAMPS (pathogen-associated molecular patterns). The discovery and characterization of the TLR family has led to a better understanding of the innate immune system. To date, 13 TLRs have been cloned in mammals (ten in humans and 12 in mice), and each seems to have a distinct function in innate immune recognition [1]. TLRs are type I transmembrane glycoproteins comprising an extracellular, transmembrane and intracellular signalling domain (Figure 1). TLRs can be classified into two groups according to their subcellular localization: TLR1, TLR2, TLR4–TLR6 and TLR11 are expressed on the plasma membrane, whereas TLR3 and TLR7–TLR9 are found in the endolysosomal compartment [2].

Human TLR4 was the first characterized mammalian Toll. TLR4 functions as a signal-transducing receptor for LPS (lipopolysaccharide) [3]. This discovery was made by positional cloning of the Lps gene in the LPS-non-responsive C3H/HeJ mouse [4]. A variety of chemically diverse PAMPS are now also known to be TLR agonists (Figure 2). For example, the major cell wall component of Gram-positive bacteria, peptidoglycan, is recognized by TLR2 [5,6]; TLR3 recognizes dsRNA (double-stranded RNA) [7]; TLR4 recognizes LPS, TLR7 recognizes ssRNA (single-stranded RNA) [8,9] and TLR9 binds unmethylated bacterial CpG DNA [10]. Engagement of TLRs by PAMPS induces the expression of pro-inflammatory cytokines that are capable of up-regulating the expression of cell adhesion molecules and chemokines, as well as increasing levels of NO and type I and II IFNs (interferons), which are directly toxic to invading micro-organisms.

In order to understand how TLRs may play a role in cardiovascular diseases, one must consider the ‘Danger Model of Immunity’ that has been proposed by Matzinger [11]. The Danger Model suggests that injured and/or stressed tissues release intracellular or extracellular danger signals. These danger signals are recognized by TLRs or other PRRs (pattern recognition receptors), leading to the activation of NF-κB (nuclear factor κB) and the production of a number of mediators known to be involved in repairing damaged tissue (Figure 2). Lending support to this hypothesis is a number of recent studies indicating that TLRs can also detect host-derived molecules. For example, Hsp (heat-shock protein) 60 and 70 have been shown to stimulate macrophages to release TNF (tumour necrosis factor) and synthesize NO in a TLR4-dependent manner [12,13]. Recent reports have also shown that hyaluronic acid, a glycosaminoglycan found in the extracellular matrix, also can activate dendritic cells by engaging TLR2 [14]. Thus stimulation by host-derived factors may serve as an intrinsic mechanism for initiating and inflammatory response after tissue injury.

### TLR SIGNALLING

The signalling pathway that is used by the TLR family of receptors is highly homologous with that of the IL-1R (IL-1 receptor) family [15]. Both TLRs and IL-1R interact with MyD88 (myeloid differentiation factor 88) in their TIR (Toll/IL-1R) domain. After TLR/IL-1R stimulation, MyD88 is recruited to the cytoplasmic TIR domain, where it facilitates the association of the IRAKs (IL-1R-associated kinases) and TRAF6
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Figure 2  Endogenous and exogenous ligands recognized by TLR2 and TLR4
LTA, lipoteichoic acid; PG, peptidoglycan; BLP, bacterial lipoproteins; LG, lipoglycans; dsDNA, double-stranded DNA.

(TNF-receptor associated factor 6). TRAF6 triggers the activation of TAKs (transforming growth factor β-activated kinases), which initiate a kinase cascade involved in activation of the IKKα (IKB (inhibitory κB) kinase α)/IKKβ/NEOM complex and phosphorylation of the inhibitory protein IκB. Phosphorylated IκB dissociates from the complex and is rapidly targeted for ubiquitination and degradation by proteasomes, causing the activation of transcription factor NF-κB, which translocates to the nucleus, mediating a number of gene transcriptions. In addition, TAK1 activates the IKK complex and MAPK (mitogen-activated protein kinase), such as ERK1/2 (extracellular-signal-regulated kinase 1/2), JNK (c-Jun N-terminal kinase) and p38 MAPK, leading to AP-1 (activator protein-1), c-Jun and c-fos activation to trigger inflammatory cytokine expression [16,17]. Although MyD88 has been reported to be involved in all TLR signalling, TLR3 seems to transduce its signals mainly through a MyD88-independent pathway, since the activation of NF-κB and MAPKs in response to dsRNA can occur in the absence of MyD88 [7]. The second major pathway emanating from TLRs depends on TRIF (TIR domain-containing adapter inducing IFN-β), also known as TICAM-1 (TIR-containing adapter molecule-1) [18,19]. TRIF plays the predominant role in TLR3-mediated NF-κB and IRF3 (IFN regulatory factor 3) activation and also promotes MyD88-independent NF-κB activation in TLR4 signalling.

TLRs AND THE CARDIAC STRESS RESPONSE TO INFECTION

Septic cardiomyopathy
Cardiac myocytes express at least four PRRs, including CD14 and TLRs 2, 3, 4 and 9 [20–24]. Studies from this and other laboratories have shown that TLR4 is critical for up-regulating the expression of TNF, IL-1β, IL-6 and NOS2 (NO synthase 2) in the heart following stimulation with LPS and that CD14 and TLR4 are essential for LPS-induced LV (left ventricular) dysfunction [20,22]. Thomas et al. [25] also demonstrated that mice deficient in IRAK1 (a downstream signalling component of TLRs) are protected from LPS-induced mortality and cardiac dysfunction. To evaluate the role of cardiac myocyte compared with leucocyte TLR4, Tavener et al. [26] studied LPS-challenged chimaeric mice with TLR4-positive leucocytes and TLR4-deficient myocytes. These mice showed reduced myocardial function in response to LPS. In contrast, chimaeric mice with TLR4-deficient leucocytes and TLR4-positive myocytes showed no response to LPS. These data suggested that TLR4 on leucocytes and not on cardiac myocytes might be
important for cardiac myocyte dysfunction in endotoxic shock. Interestingly, Binck et al. [27] reported that transplantation of wild-type mice with TLR4-deficient bone marrow did not protect against the depressant effect of endotoxin raising the possibility that other tissues, including the heart itself, could contribute to the contractile depression induced by LPS.

More recently, we have shown that TLR2 is responsible for up-regulating the expression of TNF, IL-1β and NO in the heart during Staphylococcus aureus-induced sepsis and that TLR2 mediates the LV dysfunction induced by this bacterium [23]. These studies with TLR2-deficient mice suggest that TLR2 function is important in S. aureus-mediated contractile depression, but they did not delineate in which tissue compartments TLR2 signalling is required.

Viral myocarditis

Myocarditis remains a poorly understood disease, insofar as it progresses through different phases with distinctly different mechanisms and clinical manifestations. Recently, Liu and Mason [28] have suggested that myocarditis should be viewed as a continuum that is comprised of three separate phases, namely, acute viral infection (phase I), autoimmunity (phase II) and dilated cardiomyopathy (phase III). To date, the innate immune mechanisms that contribute to host defence against viral infection of the heart remain poorly defined. Recent studies have shown that effector molecules of the innate immune system, such as TNF and NO, are beneficial to the host by virtue of their antiviral effects. Indeed, mice with defective TNF or NO expression have increased myocardial injury, a significant increase in viral titres in the heart and significantly higher mortality following EMCV (encephalomyocarditis virus) or CVB3 (coxsackievirus B3) infection [29,30]. Yasukawa et al. [31] have provided further evidence supporting the importance of innate signalling mechanisms in the pathogenesis of viral myocarditis. They demonstrated an essential role for JAK (Janus kinase) signalling in the cardiac myocyte antiviral response and a negative role of an intrinsic JAK inhibitor, the SOCS (suppressor of cytokine signalling), in the early stages of CVB3 infection. Cardiac-specific overexpression of SOCS1 inhibited enterovirus-induced of JAK/STAT (signal transducer and activator of transcription) signalling, leading to an increase in viral replication, myocardial injury and early mortality in CVB3-infected mice. More importantly, the inhibition of SOCS1 in cardiac myocytes increased cell resistance to acute cardiac injury. Thus, the extant literature suggests that innate immune mechanisms are important in the setting of acute viral infection of the heart.

TLR3 and TLR7/8 signalling can be activated by dsRNA and ssRNA respectively (Figure 3). Although the role these TLRs in the pathogenesis of viral inflammatory heart disease is yet to be determined, Fairweather et al. [32] recently reported that mice with defective TLR4 signalling had decreased CVB3 replication and myocarditis 12 days after infection when compared with wild-type mice. Cardiac levels of IL-1β and IL-18 were reduced in

Figure 3  Relationship between virus-sensing TLRs and infection of the heart

DNA Viruses

RNA Viruses

Endosome

MyD88

IRF3/7

Inflammatory Cytokines

Type I IFN

NF-κB

TRIF

Type III IFNs

TLR 9

TLR 7/8

TLR 3

Cytosol

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TLR4-deficient mice (C3H/HeJ) at these time points. It was suggested that TLR4 signalling increased IL-1β and IL-18 production, inflammation and viral replication in the heart in the latter stages of infection. Interestingly, 2 days after challenge, CVB3 titres were significantly higher in the hearts of TLR4-deficient mice when compared with wild-type littermates. Satoh et al. [33] have shown increased expression of TLR4 (in myocytes and infiltrating cells) in association with enterovirus replication in patients with myocarditis. Interestingly, enteroviral replication and high levels of TLR4 expression correlated with significantly lower systolic function. Fuse et al. [34] reported that mice deficient in MyD88 (involved in all TLR signalling except TLR3) also had less myocarditis and attenuated viral replication in the heart following infection with CVB3. Interestingly, CVB3-infected MyD88-deficient mice had significantly higher levels of IFN-α, but reduced expression of the CAR (coxsackievirus–adenovirus receptor) in the heart. The enhanced IFN expression and lower expression of CAR could explain the attenuation of disease in the MyD88-deficient mice. Studies from this laboratory have shown that infection of TLR3-knockout mice with EMCV leads to significantly earlier mortality in association with increased viral replication and myocardial injury compared with wild-type mice [21]. The differences in viral load and cardiac injury observed at 3 and 5 days after infection suggests that TLR3-mediated recognition of EMCV and subsequent activation of antiviral mechanisms control virus replication in the heart. Although mRNA levels of TNF, IL-1β, IFN-γ and IL-6 were decreased in the hearts of TLR3-knockout mice, an unexpected increase in cardiac IFN-β mRNA expression was measured in these mice after EMCV infection. Negishi et al. [35] also have documented the importance of TLR3 signalling in CVB3 infection. Interestingly, the protective effect of TLR3 against CVB3 infection was abrogated by treatment with an antibody directed against IFN-γ. Gorbea at al. [36] screened the TLR3 gene in patients diagnosed with enteroviral myocarditis/cardiomypathy. In this study, two genetic variants were identified, namely a rare non-synonymous substitution P554S in one patient with CVB3 myocarditis and a common single nucleotide polymorphism, L412F, that was detected more frequently as homozygous for phenylalanine in the patient population than in control populations. Caspase 3 cleavage at 5 days after EMCV infection (N.E. Bowles and J.G. Vallejo, unpublished work) suggests that TLR3 signalling resulted in increased TRIF-mediated induction of apoptotic signalling during viral infection could reflect a defence strategy of the host that counteracts the anti-apoptotic activities of the invading pathogen.

Viewed within the context of acute viral myocarditis, TLR expression in the heart seems to be part of an evolutionarily conserved innate immune system that is necessary to protect the heart from infectious agents. However, this protective response may have deleterious effects when cytokines are elaborated either for sustained periods of time or when cytokines are expressed at pathological levels within the heart.

**ATHEROSCLEROSIS**

The role of infection in the pathogenesis of atherosclerosis has been an area of intense study. A seroepidemiologic link between *Chlamydia pneumoniae* infection and atherosclerosis has been suggested by a number of case-control and cohort studies [38,39]. Several animal studies also have suggested that bacteria or microbial products may promote plaque growth and/or activation [40]. There is now evidence that TLR2 and TLR4 may play an important role in the development and/or progression of atherosclerotic plaques [41]. TLR4-deficient mice when crossed with ApoE (apolipoprotein E)-deficient mice have reduced atherosclerosis when compared with ApoE-deficient mice despite similar serum cholesterol levels [42]. Similar results were reported when MyD88-deficient mice were crossed with ApoE-deficient mice [43,44]. Interestingly, CD14 (a cofactor for TLR4) deficiency had no effect on development of atherosclerosis in ApoE-deficient mice.
TLR2-deficient mice also have been shown to have decreased atherosclerosis when crossed with LDL (low-density lipoprotein)-receptor-deficient mice or ApoE-deficient mice [45]. Bone marrow transplantation from TLR2-deficient to LDL-receptor-deficient mice (on a high-cholesterol diet increased susceptibility to atherosclerosis) was effective in preventing exogenous TLR2 ligand-induced disease amplification, but not baseline atherosclerotic lesion formation. This finding suggests that TLR2 expression in vascular cells plays an important role in the pathogenesis of atherosclerosis. Using en face laser scanning confocal microscopy, Mullick et al. [46] have shown that TLR2 expression is restricted to endothelial cells in regions of disturbed blood flow in LDL-receptor-deficient mice and that immune cells in murine lesions did not express TLR2. However, Edfeldt et al. [47] have shown that TLR2 and TLR4 are both expressed by macrophages and endothelial cells in human atherosclerotic plaques. Furthermore, Shinohara et al. [48] reported that expression of both TLR2 and TLR4 in the vessel wall had a synergistic effect on the development of atherosclerosis. The precise roles of TLR2 and 4 in murine models of atherosclerosis remain to be determined.

Recent clinical studies have provided evidence for a potential role of TLRs in atherosclerosis and coronary artery disease. Mizoguchi et al. [49] reported that TLR2 and TLR4 expression in monocytes correlated with the extent and severity of coronary artery diseases in patients with stable angina. Similarly, patients with unstable angina and acute myocardial infarction have also been shown to have increased number of circulating TLR4-positive monocytes [50]. In a recent prospective study, Ishikawa et al. [51] determined whether TLR2 and TLR4 also were expressed at the site of ruptured plaques in patients with acute myocardial infarction and compared local expression with circulating levels. Local TLR4 (rupture site) levels were higher than systemic levels in patients with acute myocardial infarction. In addition, TLR4 immunostaining was positive in infiltrating macrophages in ruptured plaque material. These data suggest that both expressions of systemic and plaque TLR4 may contribute to the pathogenesis of acute myocardial infarction. Additional studies will be needed to determine whether attenuating TLR signalling will be a viable option to alter the course of atherosclerosis.

A number of studies have also assessed the possible link between TLR4 polymorphisms and the risk for myocardial infarction [52–54]. Some of these studies suggested that the presence of a TLR4 polymorphism (D299G) conferred protection against coronary artery disease and carotid atherosclerosis. However, two recently published studies have not supported a relationship between the D299G TLR4 polymorphism and coronary artery disease or myocardial infarction [55,56].

**MYOCARDIAL ISCHAEMIC/REPERFUSION INJURY**

Reperfusion of blood flow to ischaemic myocardium is in and of itself associated with a distinct form of cardiac injury that is directly attributable to the toxic effects of reactive oxygen intermediates that are generated once the heart is reperfused [57]. It has been suggested that ischaemia/reperfusion leads to increase in the expression of inflammatory mediators including TNF, IL-1β, IL-6 and NO [58]. This robust inflammatory response provokes a number of deleterious effects in the heart with the most notable being LV dysfunction. Recent experimental studies suggest that TLR2- and TLR4-mediated signalling mediate the extent of LV dysfunction after myocardial ischaemia/reperfusion injury [59,60]. *In vitro* studies using cardiac myocytes have shown that hydrogen peroxide-induced oxidative stress is sufficient to increase signalling through TLR2 and that this signalling could be prevented by an anti-TLR2 antibody [61]. Sakata et al. [60] demonstrated that TLR2–TIRAP (TIR adaptor protein) signalling contributes to the development of ischaemia/reperfusion-induced LV dysfunction in the adult heart *ex vivo*. After ischaemia/reperfusion, contractile performance was significantly impaired in hearts from wild-type mice as demonstrated by a lower recovery of LV-developed pressure relative to TLR2-deficient hearts. Given that these studies were performed in isolated perfused hearts, the data suggested that the LV dysfunction was due, at least in part, to TLR2-mediated signalling in the heart. Subsequent studies using bone-marrow-derived chimaeric TLR2-deficient mice have shown that infarct size in wild-type mice with TLR2-deficient bone marrow was similar to that in TLR2-deficient mice [62]. In contrast, TLR2-deficient mice transplanted with the bone marrow of wild-type mice were not protected against myocardial ischaemia/reperfusion injury. Pretreatment with a TLR2 antagonist led to a decrease in infarct size and improved cardiac function. Furthermore, inflammation and apoptotic signalling were decreased in antibody-treated mice compared with untreated mice. These data suggested that TLR2-positive leucocytes might determine the extent of myocardial injury in this model.

Oyama et al. [63] and Chong et al. [64] also reported a smaller infarct size in TLR4 mutant mice (C3HeJ) following temporary occlusion of the left anterior descending artery. Defective TLR4 signalling was also associated with reduced neutrophil infiltration and compliment deposition in the myocardium. Hua et al. [59] also reported smaller infarcts in TLR4-deficient mice following ischaemia/reperfusion injury. Pharmacological inhibition of the PI3K (phosphoinositide 3-kinase)/Akt signalling pathway led to an increase in myocardial infarct size in TLR4-deficient mice. Interestingly, pre-treatment
with the TLR4 antagonist eritoran has also been shown to significantly reduce infarct size following myocardial ischaemia/reperfusion [65]. Additional studies will be needed to determine whether attenuating TLR2 and/or 4 signalling will be a viable option to alter the course of ischaemia/reperfusion injury.

**CARDIAC ISCHAEMIC PRECONDITIONING**

Cardiac ischaemic preconditioning occurs when brief episodes of repeated ischaemia protects the heart against a more severe and prolonged period of ischaemia followed by reperfusion. Recent studies suggest that TLR signalling may play an important homoeostatic role by delimiting tissue injury following ischaemia/reperfusion injury in several models. Izuishi et al. showed that pretreatment with HMGB1 (high-mobility group box protein 1) (a TLR4 ligand) conferred cytoprotection in liver ischaemia/reperfusion injury in wild-type mice but not in TLR4 mutant mice [66]. Furthermore, treatment with a TLR2-specific ligand decreased brain infarct size in mice that were subjected to focal cerebral ischaemia/reperfusion injury [67]. Dong et al. [68] have shown that repetitive injury to the heart, in the form of short bouts of ischaemia followed by reperfusion, confers cytoprotection through TLR2–TIRAP-dependent signalling pathways. Ischaemic preconditioning resulted in a significant increase in the percentage recovery of LVDP (LV developed pressure) in wild-type mouse hearts, but not in TIRAP-deficient mouse hearts after ischaemia/reperfusion injury. Ischaemic preconditioning resulted in a significant increase in cardiac function in TLR4-deficient hearts but not in TLR2-deficient hearts. The TLR2 agonist Pam3CSK4 mimicked the effects of ischaemic preconditioning in wild-type but not TLR2-deficient mice. Mersmann et al. [69] also have shown that preconditioning by Pam3CSK4 reduces infarct size, reduces troponin T release and improves cardiac function in mice subjected to ischaemia/reperfusion. This was associated with decreased leukocyte infiltration of the ischaemic area as a result of decreased CXCL10 (CXC chemokine ligand 10) expression. These data suggest that the innate immune system may confer short-term beneficial effects in the heart by activating cytoprotective-signalling pathways via TLR2 signalling.

**HEART FAILURE AND MYOCARDIAL REMODELLING**

The link between heart failure and inflammation was first reported by Levine et al. [70]. These investigators noted that levels of TNF were elevated in the setting of heart failure. A number of studies have now shown that, in addition to TNF, other pro-inflammatory cytokines and chemokines are also involved in the progression of heart failure [71,72]. Recent clinical and experimental studies suggest that TLRs may play an important role in the development and progression of heart failure. Frantz et al. [73] were the first to report the expression of TLR4 in human and rodent heart. Increased myocardial TLR4 expression was noted in tissue sections from hearts of humans with ischaemic cardiomyopathy and of rodents with experimental heart failure. Using TLR2-deficient mice, Shishido et al. [74] reported a significant reduction in mortality and LV dysfunction after coronary artery ligation. Despite similar infarct size, TLR2-deficient mice exhibited less ventricular remodelling when compared with wild-type mice. Histological examinations demonstrated that myocardial fibrosis was decreased significantly in the non-infarct area in TLR2-deficient mice. This finding was associated with reduced expression of TGF-β1 and collagen type 1 in hearts of TLR2-deficient mice. Timmers et al. [75] also reported that TLR4 mutant mice had reduced LV remodelling and preserved LV function after myocardial infarction. Interstitial fibrosis and myocardial hypertrophy were reduced in the non-infarct area in TLR4 mutant mice. The expression of inflammatory cytokines was decreased, and collagen density was increased in the infarct area in TLR4 mutant mice. These experimental data suggest that TLR2 and TLR4 may be viable targets in the treatment of ischaemic heart failure.

There is some evidence that TLRs may play a role in clinical heart failure. Birks et al. [76] reported an increase in both TLR4 and the IL-1 receptor in the myocardium of patients with deteriorating heart function who subsequently required LV assist device implantation. Patients with dilated cardiomyopathy had greater TLR4 mRNA expression compared with those with ischaemic heart disease. Recently, Mann et al. [77] examined the activation of innate immunity genes in patients with heart failure due to dilated cardiomyopathy, ischaemic cardiomyopathy and viral cardiomyopathy. They reported that expression levels of TLR2 and TLR4 were decreased in explanted hearts from patients with all three forms of cardiomyopathy. Interestingly, the expression levels of the downstream mediators TIRAP and IRAK-4 were increased in these patients. These data suggest that a maladaptive innate immune response may contribute to the pathogenesis of human heart failure through sustained expression of pro-inflammatory mediators.

**CONCLUSIONS**

The present review summarizes recent studies that suggest that TLRs play an important role in orchestrating homoeostatic responses within the heart. However, the short-term beneficial effects of TLR signalling may be lost if myocardial expression becomes dysregulated (i.e. septic cardiomyopathy), in which case, the beneficial effects of these proteins may be negated by their known deleterious effects. If the fundamental role of TLRs...
is to protect the heart against and/or delimit tissue injury during infection (i.e., viruses), why then is the expression of TLRs within the heart often associated with maladaptive inflammatory reactions that lead to increased tissue damage in the presence of non-infectious stimuli? Thus, from an evolutionary perspective, it is possible that Nature may have conserved molecules that conferred short-term benefits in the host, but that also had the potential for long-term detrimental effects.

The question that remains unanswered is whether it will be possible to modulate the inappropriate/maladaptive consequences of TLR activation in the mammalian heart, while preserving the important advantages they provide to the host. There have been some clinical trials with agents that antagonize TLR signalling. A Phase 2 trial of the drug E5564 (eritoran) in patients with sepsis has been completed, and treatment was well tolerated [78]. The observed trend towards a lower mortality, in patients with severe sepsis and a high predicted risk of mortality, is being investigated in a Phase 3 clinical trial. A randomized, double-blind, placebo-controlled trial (293 patients) of TAK-242 (TLR4 antagonist) for the treatment of severe sepsis did not show differences in mortality [79]. Similar studies for the prevention or treatment of heart disease do not exist. Given that inhibition of innate immune molecules (i.e. TNF) have failed in the treatment of heart failure [80], it will be essential to fully characterize the role of TLRs in normal cardiac physiology prior to disrupting their function in cardiovascular diseases.

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