Toll-like receptors and myocardial contractile dysfunction

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Toll-like receptors (TLRs), vertebrate homologues of the drosophila Toll receptor, have been shown to play essential roles in the innate immune response.1 As TLRs recognize pathogen-associated molecular patterns (PAMPs), they are also known as pattern recognition receptors (PRRs). So far, 11 members of human TLRs have been identified.1 Roles of TLRs have been vigorously studied also in the area of cardiovascular sciences, and these molecules are responsible for the genesis of a variety of cardiovascular disorders, including myocardial dysfunction during sepsis, ischaemia/reperfusion, heart failure, cardiac hypertrophy, and atherosclerosis.2–4 Among the TLRs, TLR2 and TLR4 have been investigated most extensively. TLR2 recognizes gram-positive bacterial lipopolysaccharides and peptidoglycans, and lipopolysaccharide, endotoxin, is one of the PAMPs for TLR4. These TLR2 and TLR4 are shown to contribute to myocardial contractile dysfunction during sepsis. Activation of inflammatory cytokines and nuclear factor kB (NF-κB) as well as augmented expression of inducible nitric oxide synthase (iNOS) are the major consequences of the stimulation of these TLRs. Expression of TLR4 was augmented in the myocardium form patients with heart failure,5 and TLR4 was involved in the genesis of myocardial hypertrophy.6 On the other hand, both TLR2 and TLR4 are assumed to be involved in the development and progression of atherosclerosis, and some clinical studies have linked gene polymorphisms of these TLRs to the incidence of atherosclerotic diseases such as myocardial infarction.4

The roles of the other members of TLRs in cardiovascular diseases, however, are not completely understood. A recent study7 has shown that TLR2, TLR3, TLR4, TLR5, TLR7, and TLR9 are constitutively expressed in the murine heart tissues as well as a cardiomyocyte cell line. In this study, ligand activation of TLR2, TLR4, or TLR5 led to contractile dysfunction of the isolated murine cardiomyocytes, along with augmented expression of inflammatory cytokines via NF-κB activation. In contrast, activation of the other TLRs failed to induce any significant responses. Although it is well known that bacterial oligodeoxynucleotides (ODN) containing unmethylated deoxycytidyl-deoxyguanosine (CpG) motifs (ODN–CpG) can cause septic shock via TLR9,8 the significance of TLR9 in myocardial inflammation and depression during sepsis remain to be determined.

In this issue of the Cardiovascular Research, Knuefermann et al.9 have elegantly demonstrated significant roles of TLR9 in myocardial contractile dysfunction during sepsis. They isolated cardiac myocytes from wild-type and TLR9-deficient mice and stimulated these cells with CpG–ODN. The bacterial DNA was taken up into the cytoplasm and nucleus of the isolated cardiomyocytes. The CpG–ODN augmented myocardial expression and plasma levels of inflammatory cytokines (TNFα, IL-1β, and IL-6) only in the wild-type mice. After the exposure to CpG–ODN, contraction of the isolated cardiac myocytes from the wild-type mice was significantly suppressed, whereas that of the myocytes from the TLR9-deficient mice remained unchanged. Thus, these data suggest that bacterial DNA exerts a negative inotropic effect via activation of TLR9. In this study, CpG–ODN induced activation of NF-κB and expression of iNOS in the wild-type mice, but not in the TLR9-deficient mice, suggesting that NF-κB and iNOS may also be key molecules in the TLR9-dependent myocardial contractile dysfunction. This study is the first report that has successfully confirmed the functional significance of TLR9 in the genesis of cardiovascular disorders.

As discussed above, multiple TLRs may be involved in myocardial inflammation and contractile dysfunction. In addition, NF-κB may play a key role in the TLR-dependent signalling pathways. Signalling pathways converging from individual TLRs on NF-κB may be complex and worth investigating. Many intracellular molecules, such as MyD88, TRAF6, and TAK1, are involved in these pathways.3,4 The interactions among TLRs or the relative importance of individual TLRs should be clarified in specific cardiovascular pathologies, because such an approach will help us to determine which TLR is a main therapeutic target in each disease state. In particular, the interaction between TLR4 and TLR9 may be important in the genesis of myocardial
contractile dysfunction. Furthermore, the significant role of iNOS in the genesis of cardiac contractile dysfunction has also been confirmed in the current study.9 The link between TLRs and iNOS may be the basis for the evolutionarily conserved myocardial effects of iNOS,10 although evolutionary significance of this link in the innate and adaptive immune responses remain to be elucidated. The concept of ‘the heart as an immunological organ’3 should be examined in this context.

The above-mentioned role of TLR9 seems to have some clinical implications. Septic shock is still one of the most serious conditions with very high mortality rates. Even in a recent clinical trial,11 survival rates of patients with septic shock who were treated with standard regimens using catecholamines were no more than 50% within 90 days. Thus, more effective therapies for myocardial dysfunction during septic shock are desirable. Many researchers have tried to develop specific agonists or antagonists for TLR molecules as new-age drugs against infectious and autoimmune diseases. Some synthetic oligonucleotides containing the CpG motifs have been developed as TLR9 agonists or antagonists, and have been studied as therapies for cancer, hepatitis, allergy, asthma, and autoimmune diseases.12 An experimental study has reported that oligonucleotide inhibitors were effective for CpG-ODN-induced lung injury.13 Thus, TLR9 antagonists may be useful for treating haemodynamic compromise in some patients with septic shock.

Considering the theory that cytokine activation and iNOS expression also play significant roles in usual types of heart failure, TLR9 antagonists may further be applied to treatment of human heart failure in general. An earlier study has reported that the serum levels of endotoxin were elevated in patients with heart failure, suggesting that endotoxin can be absorbed through the oedematous intestine with increased permeability.14 Bacterial DNA can also enter the human bloodstream via various routes, and may trigger TLR9-dependent signalling pathways and eventually cause myocardial contractile dysfunction, although it is actually difficult to detect such DNA molecules. Therefore, TLR9 blockade may be another effective strategy for treatment of heart failure, particularly when activation of immune responses is suspected to be sustained. The partial nature of the TLR9 blockade may be beneficial because basic activity of the innate immune system operated by other TLRs may be preserved during the treatment.

References