Toll-like receptors: emerging therapeutic targets for heart failure

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Abstract

Toll-like receptors (TLRs) have been identified as central innate immune receptors. They distinguish among different patterns of pathogens and rapidly activate an innate immune response. However, TLRs can also be stimulated by host-derived molecules. They are expressed in the cardiovascular system and could thus be a key link between cardiovascular diseases and the activation of the immune system. Good experimental evidence is now available suggesting that TLR signaling promotes injury in the heart in response to ischemia, ischemia-reperfusion or hypertrophic stimuli.
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Keywords: Heart failure, innate immunity, ischemic injury, myocardium, Toll-like receptors (TLR)

Introduction

At first glance, pathologic mechanisms in cardiac diseases seem far from involving pathogen defense mechanisms. However, several lines of evidence link the activation of the immune system with cardiovascular diseases. For example, in patients and animal models with heart failure, the immune system is robustly activated, although there is no evidence for a specific pathogen in its etiology. Activation of the immune system is associated with unfavorable outcome, and experimental studies suggest it also has a role in adverse cardiac remodeling and survival after myocardial infarction. Most of the activated immune mechanisms are part of the innate immune system, an evolutionarily ancient, non clonal immune recognition and effector system present in both invertebrates and vertebrates, and distinct from adaptive immunity, which has evolved only in vertebrates.

Toll like receptors (TLRs) have emerged as central receptors of the innate immune system. To date, 11 human and 13 mouse TLRs have been cloned [1]. The ligands for TLRs are molecular motifs produced by pathogens, not by the host; for example, TLR4 recognizes cell wall components of Gram-negative bacteria. However, they can also be activated by host-derived molecules released upon events such as tissue injury. For example, heat-shock protein 60 (HSP-60), a molecular chaperone conserved in both invertebrates and vertebrates, can activate nuclear factor kappa B (NF-κB) through both TLR2 and TLR4 [2]. Thus these endogenous TLR ligands may activate the innate immune response in cardiovascular diseases such as ischemic cardiac injury, thereby explaining immune activation in a primary non immune disease.

Toll-like receptor signaling

TLR signaling is complex (*Figure 1*) and beyond the scope of this article (for a detailed description see [3]).

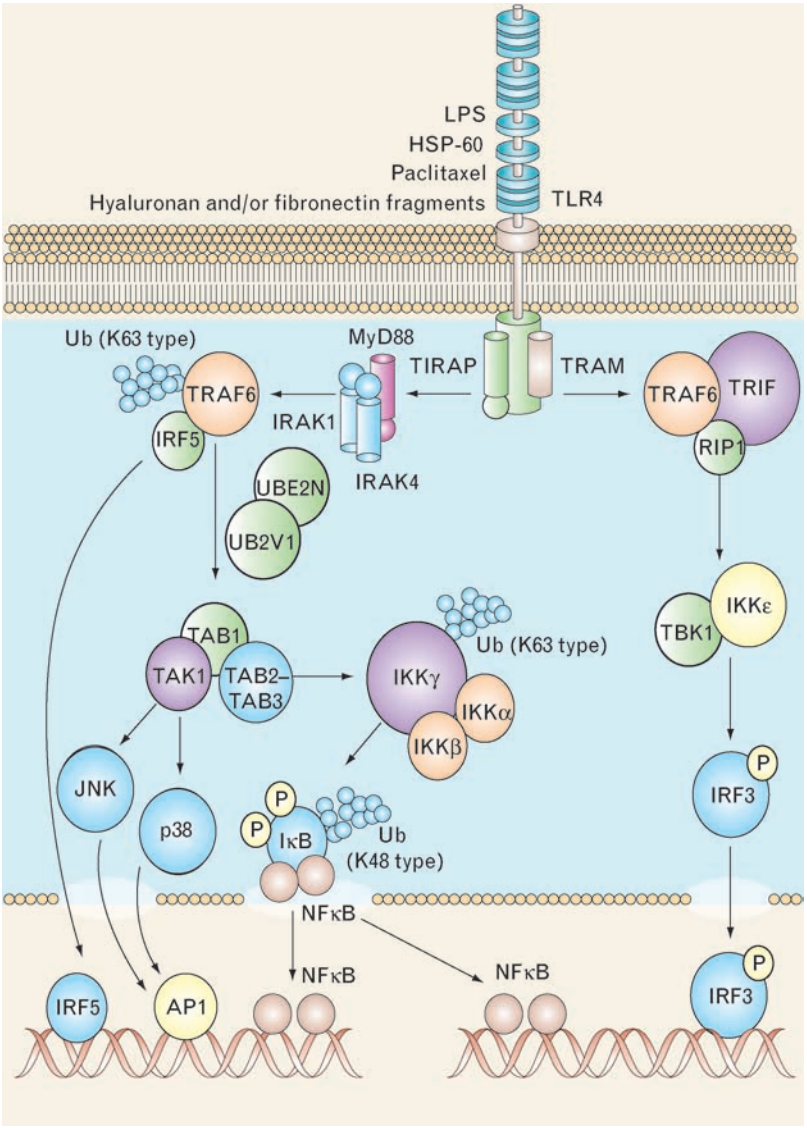

Figure 1. Summary of the Toll-like receptor signaling pathway. AP1, activator protein-1; HSP-60, heat-shock protein 60; IκB, inhibitory protein kappa B; IKKα, β, γ, ε, IκB kinases α, β, γ, and ε; IRAK1, IRAK4, interleukin receptor-associated kinases 1 and 4; IRF3, IRF5, interferon regulatory factors 3 and 5; JNK, Jun kinase; LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response gene 88 protein; NFκB, nuclear factor kappa B; P, inorganic phosphate; p38, mitogen-activated protein kinase p38; RIP1, receptor-interacting protein 1; TAB1, TAB2, TAB3, TAK1-binding proteins 1, 2, and 3; TAK1, transforming growth factor-β-activated kinase 1; TBK1, TANK (TRAF family member-associated NFκB activator) binding kinase 1; TIRAP, Toll-interleukin 1 receptor (TIR) domain-containing adaptor protein; TLR4, Toll-like receptor 4; TRAF6, tumor necrosis factor receptor-associated factor 6; TRAM, TRIF-related adapter molecule; TRIF, TIR1 domain-containing adapter-inducing interferon-β; Ub (K63 and K48 types), lysine 63- and 48-linked ubiquitins; UB2V1, ubiquitin-conjugating enzyme E2 variant 1; UBE2N, ubiquitin-conjugating enzyme E2N. (Reproduced from Frantz et al [4] with permission.)

Signaling converges on the activation of the transcription factor, NF-κB.

Toll-like receptors in heart failure

In addition to the role of TLR signaling in atherosclerosis, which has been the focus of other reviews [4,5] and is beyond the scope of this article, several reports suggest a regulation of TLRs in patients with ischemic heart disease [6]. For example, an increase in circulating TLR2- or TLR4-positive monocytes has been observed in unstable angina, acute myocardial infarction, and chronic heart failure [7,8]. Activation of TLR4 in monocytes is associated with the development of heart failure after acute myocardial infarction [9]. In patients with ST-segment elevation myocardial infarction, activated TLR4 is independently predictive of 30-day major adverse clinical outcome [10].

Whereas the clinical evidence is only indirect, direct hints for a role of TLRs in ischemic heart disease come from in-vitro and in-vivo data. Indeed, TLRs are readily detectable in cardiac myocytes. TLRs 2, 3, 4, 6, 7 and 9 are expressed in ventricular myocytes,

whereas TLR1 and 5 are not [11–13]. In vitro, a potential role of TLR2 in the response to oxidative stress has been established in neonatal rat cardiac myocytes. Blockade of TLR2 function was found to inhibit hydrogen peroxide-induced activation of NF-κB and diminished cytotoxicity and apoptosis [11]. In contrast, TLR4 activation can reduce apoptosis of cardiac myocytes, an effect mediated by nitric oxide synthase 2 [14]. Activation of TLRs 2 and 4 reduces myocyte contractility and cytokine secretion in HL-1 cells, an immortalized cell line with adult cardiac myocyte properties [13]. This suggests that TLR signaling may be important for myocardial diseases.

In vivo, TLRs and their signaling components are upregulated in experimental or clinical heart failure. Expression of TLR4 is increased in the myocardium of patients with advanced heart failure [12,15]. In addition, there is a change in the pattern of expression of TLR: whereas in normal murine and human myocardium, TLR4 expression is diffuse and predominantly confined to cardiac myocytes, myocardium from patients with advanced heart failure displays focal areas of intense TLR4 staining (*Figure 2*). The reason for this change in expression of TLR4 in the remodeled failing myocardium is not yet known [12]. TLR signaling converges on the activation of interleukin receptor-associated kinase 1 (IRAK1) and the transcription factor NF-κB (*Figure 1*). In line with the previous findings, both IRAK1 and NF-κB are activated by cardiac ischemia or in experimental and human heart failure [16–18]; NF-κB is also increased in peripheral leukocytes of patients with stable heart failure [19]. Taken together, the evidence is strong that TLR and its signaling components are activated by ischemic heart failure.

The coronary artery ligation model is the best established and clinically most relevant experimental model of heart failure. After coronary artery ligation, mortality and left ventricular dilatation were significantly reduced, and left ventricular function was preserved in TLR2$^{-/-}$ mice compared with wild-type mice. These effects may be mediated by TLR-dependent changes in extracellular matrix remodeling [20]. Furthermore, left ventricular remodeling and

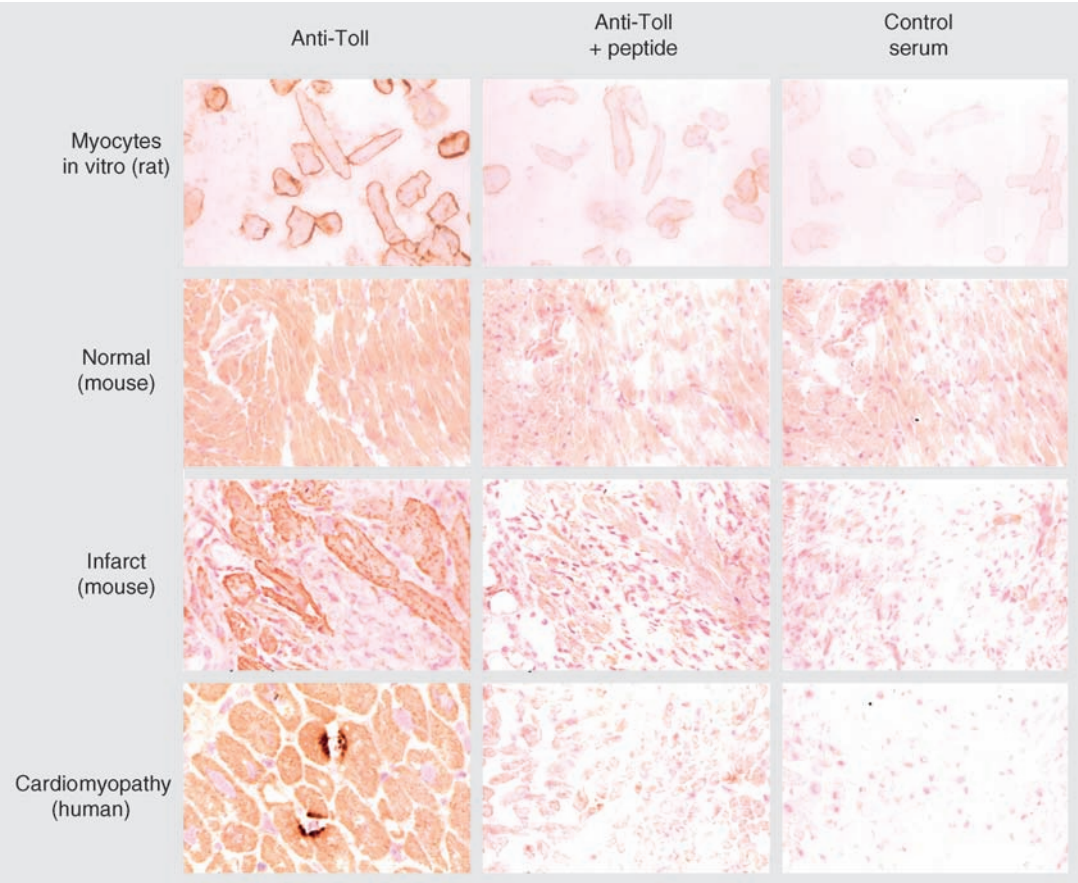

Figure 2. Toll-like receptor 4 (TLR4) in myocytes from rat, murine, and human myocardium 24 h after isolation and staining with a polyclonal anti-Toll antibody. Primary isolates of adult rat ventricular myocytes targeted to a TLR4-specific epitope adjacent to the cytoplasmic Toll interleukin 1 receptor (TIR) domain of human TLR4 (upper panel). Normal murine cardiac muscle (magnification 200×; second panel) exhibited diffuse, homogeneous myocyte staining. However, murine cardiac myocytes adjacent to an area of ischemic injury induced by coronary artery ligation exhibited intense sarcolemmal TLR4 staining (third panel). Finally, cardiomyocytes from humans with dilated cardiomyopathy (lower panel) displayed intensely stained focal expression of TLR4. (Reproduced from Frantz et al [12], with permission.)

survival are improved in TLR4-deficient mice, together with a reduction in proinflammatory cytokines, alterations of extracellular matrix remodeling, but no change in the rate of apoptosis [21,22].

In parallel, mice with targeted deletion of the NF-κB subunit, p50, are protected from left ventricular dilatation after myocardial infarction and have preserved left ventricular function. Collagen content and expression of matrix metalloproteinase-9 are significantly lower in p50-knockout mice after myocardial infarction and may account for improved left ventricular remodeling [16]. Thus TLRs and their downstream signaling components are important in left ventricular remodeling after myocardial infarction.

Conclusion

Toll-like receptors are an important family of innate pattern recognition receptors that trigger the activation of an immune response. TLRs are readily detectable in the cardiovascular system and upregulated in ischemic cardiac diseases. There is good experimental evidence that inhibition of TLR2 and TLR4 signaling could reduce cardiac damage after ischemic injury. Unfortunately, a transfer of these results to the clinic is hampered by the fact that an intact immune system is necessary for many protective pathways. However, prolonged immune activation may also activate unfavorable signal cascades that drive disease progression. Thus further research in this field is necessary to find a consensus as to the specific role played by the innate immune system and the potential therapeutic impact in these diseases.

Summary

TLRs, central receptors of the innate immune system, are expressed in the heart and activated in experimental and human heart failure. TLR activation is associated with adverse outcome. Inhibition of TLR2 and TLR4 improves left ventricular remodeling after experimental myocardial infarction. The therapeutic role of TLR inhibition in humans remains to be defined. ■

REFERENCES

1. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. 2006;124:783–801.
2. Vabulas RM, Ahmad-Nejad P, da Costa C, et al. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem*. 2001;276:31332–31339.
3. Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys Res Commun*. 2009;388:621–625.
4. Frantz S, Ertl G, Bauersachs J. Mechanisms of disease: Toll-like receptors in cardiovascular disease. *Nature Clinical Practice*. 2007;4:444–454.
5. Michelsen KS, Arditi M. Toll-like receptor signaling and atherosclerosis. *Curr Opin Hematol*. 2006;13:163–168.
6. Frantz S, Ertl G, Bauersachs J. Toll-like receptor signaling in the ischemic heart. *Front Biosci*. 2008;13:5772–5779.
7. Methe H, Kim JO, Kofler S, Weis M, Nabauer M, Koglin J. Expansion of circulating Toll-like receptor 4-positive monocytes in patients with acute coronary syndrome. *Circulation*. 2005;111:2654–2661.
8. Foldes G, von Haehling S, Okonko DO, Jankowska EA, Poole-Wilson PA, Anker SD. Fluvastatin reduces increased blood monocyte Toll-like receptor 4 expression in whole blood from patients with chronic heart failure. *Int J Cardiol*. 2008;124:80–85.
9. Satoh M, Shimoda Y, Maesawa C, et al. Activated toll-like receptor 4 in monocytes is associated with heart failure after acute myocardial infarction. *Int J Cardiol*. 2006;109:226–234.
10. Sheu JJ, Chang LT, Chiang CH, et al. Prognostic value of activated toll-like receptor-4 in monocytes following acute myocardial infarction. *Int Heart J*. 2008;49:1–11.
11. Frantz S, Kelly RA, Bourcier T. Role of TLR-2 in the activation of nuclear factor-kappa B by oxidative stress in cardiac myocytes. *J Biol Chem*. 2001;276:5197–5203.
12. Frantz S, Kobzik L, Kim YD, et al. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. *J Clin Invest*. 1999;104:271–280.
13. Boyd JH, Mathur S, Wang Y, Bateman RM, Walley KR. Toll-like receptor stimulation in cardiomyoctes decreases contractility and initiates an NF-kappaB dependent inflammatory response. *Cardiovasc Res*. 2006;72:384–393.
14. Zhu X, Zhao H, Graveline AR, et al. MyD88 and NOS2 are essential for Toll-like receptor 4-mediated survival effect in cardiomyocytes. *Am J Physiol Heart Circ Physiol*. 2006;291:H1900–H1909.
15. Birks EJ, Felkin LE, Banner NR, Khaghani A, Barton PJ, Yacoub MH. Increased toll-like receptor 4 in the myocardium of patients requiring left ventricular assist devices. *J Heart Lung Transplant*. 2004;23:228–235.
16. Frantz S, Hu K, Bayer B, et al. Absence of NF-kappa B subunit p50 improves heart failure after myocardial infarction. *FASEB J*. 2006;20:1918–1920.
17. Tillmanns J, Carlsen H, Blomhoff R, et al. Caught in the act: in vivo molecular imaging of the transcription factor NF-kappaB after myocardial infarction. *Biochem Biophys Res Commun*. 2006;342:773–774.
18. Chao W, Shen Y, Li L, Rosenzweig A. Importance of FADD signaling in serum deprivation- and hypoxia-induced cardiomyocyte apoptosis. *J Biol Chem*. 2002;277:31639–31645.
19. Frantz S, Stoerk S, Ok S, et al. Effect of chronic heart failure on nuclear factor kappa B in peripheral leukocytes. *Am J Cardiol*. 2004;94:671–673.
20. Shishido T, Nozaki N, Yamaguchi S, et al. Toll-like receptor-2 modulates ventricular remodeling after myocardial infarction. *Circulation*. 2003;108:2905–2910.
21. Timmers L, Sluijter JP, van Keulen JK, et al. Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function following myocardial infarction. *Circ Res*. 2008;102:257–264.
22. Riad A, Jager S, Sobirey M, et al. Toll-like receptor-4 modulates survival by induction of left ventricular remodeling after myocardial infarction in mice. *J Immunol*. 2008;180:6954–6961.