

REVIEW

Inappropriate Activation of TLR4/NF- κ B is a Cause of Heart Failure

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Abstract

Significance: Heart failure, a disease with extremely high incidence, is closely associated with inflammation and oxidative stress. The Toll-like receptor 4 (TLR4)/nuclear factor kappa-B (NF- κ B) pathway plays an important role in the occurrence and development of heart failure.

Recent advances: Previous studies have shown that TLR4/NF- κ B causes heart failure by inducing oxidative stress and inflammation; damaging the endothelia; promoting fibrosis; and inducing myocardial hypertrophy, apoptosis, pyroptosis, and autophagy.

Critical issues: Understanding the pathogenesis of heart failure is essential for the treatment of this disease. In this review, we outline the mechanisms underlying TLR4/NF- κ B pathway-mediated heart failure and discuss drugs that alleviate heart failure by regulating the TLR4/NF- κ B pathway.

Future directions: During TLR4/NF- κ B overactivation, interventions targeting specific receptor antagonists may effectively alleviate heart failure, thus providing a basis for the development of new anti-heart failure drugs.

Keywords: TLR4; NF- κ B; heart failure; oxidative stress; inflammation

Introduction

Heart failure is a disease caused by ventricular ejection or filling difficulty due to various structural or functional lesions of the heart, thus resulting in systemic organ hypoperfusion. According to large epidemiological studies, the incidence of heart failure

in adults has reached 1–2% [1–3] and exceeds 10% in people 70 years of age or older [4]. Various factors, such as myocardial hypoxia, inflammation, endothelial damage, and increased cardiac load, can lead to heart failure. Some of these factors can form a vicious cycle that further promotes heart failure; for example, myocardial hypoxia can cause heart failure, which in turn aggravates myocardial hypoxia. Therefore, understanding the pathogenesis of heart failure is important to develop new therapeutic options. Toll-like receptor (TLR) 4, a member of the TLR family, is a classical pattern recognition receptor (PRR), and its downstream

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transcription factor nuclear factor kappa-B (NF- κ B) plays an important role in the occurrence and development of inflammation and oxidative stress [5, 6]. The TLR4/NF- κ B signaling pathway is activated by signals such as lipopolysaccharide (LPS) and reactive oxygen species (ROS), and subsequently induces downstream inflammation and oxidative stress, thereby exerting a variety of effects on heart structure and function.

Overview of the Toll-Like Receptor Family And Composition of the TLR4/NF- κ B Signaling Pathway

TLRs are PRRs that are widely present across many species and recognize external stimuli, such as pathogenic microorganisms, as well as internal stimuli, such as tumors or cell damage, and play important roles in the immune response [7]. Ten TLRs have been identified in humans, among which TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are located on the plasma membrane, and TLR3, TLR7, TLR8, and TLR9 are expressed in the cytoplasm. The signal transduction of TLRs occurs mainly through myeloid differentiation factor 88 (MyD88) and Toll/Interleukin-1 receptor domain-containing adaptor-inducing interferon- β (TRIF). Some differences are observed among TLR subtypes: all TLRs except TLR3 require MyD88 to be recruited to the TIR domain. TLR3 and TLR4 ligands recruit the linker protein TRIF, whereas TLR2 and TLR4 signals require not only MyD88 but also Mal/TIRAP cooperation [8, 9].

TLR4 is an important member of the TLR family and is one of the most widely studied receptors in the current literature. Its natural ligand, LPS, is a strong stimulant that induces severe inflammatory reactions. MyD88-dependent and TRIF-dependent pathways are involved in signal transduction in cells after TLR4 stimulation. In the MyD88-dependent pathway, TLR4 and myeloid differentiation protein 2 form a dimer after binding their ligand; the dimer in turn binds the TIR domain of MyD88, thus forming an active TLR4/MyD88 complex. The complex then activates IL-1R-associated kinase 4 (IRAK4), IRAK1, IRAK2, and tumor necrosis factor receptor-associated factor 6 (TRAF6), thereby inducing inhibitory kappa B (I κ B) kinase (I κ K) and

finally activating the NF- κ B pathway [10, 11]. The MyD88-independent pathway of TLR4 activation, i.e., the TRIF-dependent pathway, is closely associated with the expression of interferons.

The NF- κ B pathway is essential in the inflammatory response and has important roles in various inflammatory conditions. Currently, five NF- κ B proteins are known: p50, p65, p52, RelB, and c-Rel [12]. In the absence of external stimulation, the NF- κ B protein p50/p65 binds the inhibitory protein I κ B and forms an inactive trimer in the cytoplasm. When external stimuli act on the corresponding receptors, for example, when LPS activates TLR4, NF- κ B is activated through the classical pathway, which involves activation of I κ K and subsequent phosphorylation of I κ B. Consequently, phospho-I κ B dissociates from the trimer, thus leading to p50/p65 translocation to the nucleus and specific binding to the κ B gene, thus causing widespread expression of inflammatory factors and an inflammatory response. In addition, upregulated inflammatory factors further activate NF- κ B, thereby forming a positive feedback loop [13]. Among them,

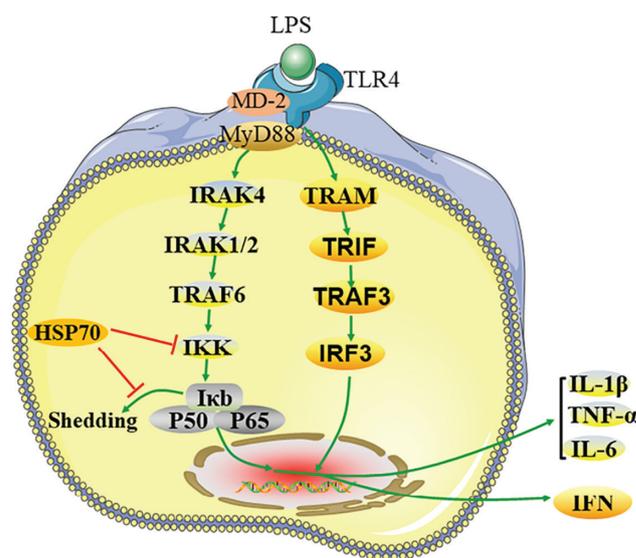


Figure 1 TLR4 and its downstream pathways – MyD88 pathway.

After being activated by ligands, TLR4 and MD2 form a dimer that recruits MyD88, which in turn activates downstream IRAK4, IRAK1/2, TRAF6, I κ K, and NF- κ B, and then promotes the expression of IL-1 β and other inflammatory cytokines. TRIF pathway: after ligand binding, TLR4 activates TRAM, TRIF, TRAF3, and IRF3 in sequence, and is closely associated with the expression of IFN. Intracellular HSP70 inhibits the activation of I κ K and inhibits I κ B shedding, thus blocking NF- κ B pathway activation.

inflammatory factors such as IL-1 β and tumor necrosis factor (TNF)- α , produced after the activation of NF- κ B, have strong chemotactic effects on neutrophils and cause inflammatory cell infiltration (Figure 1).

Molecular Links between TLR4/NF- κ B and Heart Failure

Oxidative Stress

Oxidative stress is a state of imbalance between oxidation and reduction, mainly involving ROS. ROS are not exclusively harmful, but in fact are necessary for normal cell function and survival. However, excessive ROS can lead to oxidative stress [14], which plays an important role in the occurrence and development of heart failure. Excessive ROS production and consequent oxidative stress lead to the oxidation of biomolecules, such as lipoproteins and deoxyribonucleic acid. Sources of ROS in human tissues include the mitochondrial electron transport chain, NADPH oxidase, xanthine oxidase, uncoupled nitric oxide synthase (NOS), and the arachidonic acid metabolic pathway. Among these, ROS in the heart are derived mainly from the mitochondrial electron transport chain, NADPH oxidase, and xanthine oxidase [15]. For heart diseases with different etiologies, the main sources of ROS vary slightly; for example, in diabetic cardiomyopathy, an important source of ROS accumulation in myocardial cells is the increase in fatty acid metabolism, because the accumulation of free fatty acids may damage mitochondria and lead to their excessive oxidation [16, 17].

The activation of TLR4 and its downstream effector NF- κ B inhibits the expression of cardiac mitochondrial respiratory chain complexes II, III, IV, and V, thus leading to mitochondrial respiratory chain dysfunction and ROS production [18]. The increase in oxidative stress levels after TLR4 activation is also associated with a decrease in endogenous antioxidant substances such as catalase and glutathione [18]. The activation of TLR4/NF- κ B induces the expression of ROS, but ROS also act on TLR4 and activate NF- κ B, thus stimulating the expression of inflammatory factors [19]. Moreover, ROS-induced TLR4 and downstream

NF- κ B activation can cause sympathetic overactivity and fatal ventricular arrhythmia after myocardial infarction [20]. The influence of ROS on the NF- κ B pathway lies not only in NF- κ B dimer molecules but also in upstream molecules such as I κ K; thus, ROS regulate the entire NF- κ B pathway [21]. ROS not only activate NF- κ B in diabetic cardiomyopathy, but also activate NF- κ B and caspases in the stress response to high osmotic pressure, thus leading to the death of myocardial cells [22]. The occurrence of myocardial damage in Barth syndrome is also associated with ROS production [23]. Because myocardial damage caused by ROS is harmful, measures must be taken to prevent and treat its pathogenesis. Studies have shown that antioxidants alleviate the myocardial damage caused by ROS. For example, reducing substances, such as astragaloside IV and salvianolic acid, have been found to ameliorate myocardial damage by inhibiting TLR4/NF- κ B [24–26].

Inflammation

Inflammation is a normal reaction of the body and a powerful means of resisting pathogen invasion and repairing normal tissues. However, excessive and long-term chronic inflammation can cause serious damage to the body [27], such as myocardial infarction, hypertension, and atherosclerosis [28–30], all of which contribute to heart failure.

TLR4 and the NF- κ B pathway participate in the occurrence and development of inflammation, and are closely associated with the progression of heart failure. TLR4 is the most expressed TLR in myocardial tissue [31], and its expression significantly increases in cardiomyocytes exposed to ischemia [32]. Moreover, when intestinal microorganisms enter the circulation, LPS activates TLR4, thereby stimulating systemic inflammation and causing adverse cardiovascular events. When drugs are used to decrease serum LPS, the myocardial infarct size also significantly decreases [33]. These studies have demonstrated that activation of TLR4 and its downstream pathways is important in inflammation-induced heart injury. In TLR4 gene knockout mice, compared with wild-type mice, a smaller decrease in heart function is observed during acute hypoxia, and cardiac function is less affected by LPS [34]. In rats treated with the TLR4-specific inhibitor

TAK-242, compared with a control, the myocardium exhibits a weaker inflammatory response and milder myocardial damage after the formation of coronary microembolization. These effects are achieved primarily through inhibition of TLR4/MyD88/NF- κ B signaling, thus decreasing the activation of NLR family pyrin domain containing 3 (NLRP3) inflammasomes [35]. These results indicate that TLR4 and downstream NF- κ B expressed in myocardial cells play key roles in acute cardiac failure. More importantly, serum IL-1 β and TNF- α significantly increase, and the cardiac TLR4/MyD88/NF- κ B pathway is activated after exposure to a single long-term stress; significant increases in protein levels of atrial natriuretic peptide and brain natriuretic peptide have also been observed in the heart tissue in rats [36].

Targeted measures are necessary to address the negative role of TLR4/NF- κ B in inflammation and its effects on cardiac function. A study has demonstrated that infusion of the anti-inflammatory cytokine IL-10 inhibits the NF- κ B pathway, reverses isoproterenol-induced ventricular remodeling, and significantly improves left ventricular function [37]. Moreover, IL-10 counteracts TNF- α and maintains the balance between anti-inflammation and pro-inflammation in the heart [38].

Intracellular HSP70, a Tool to Regulate TLR4/NF- κ B

Heat shock proteins (HSPs) are molecular chaperones with important roles in maintaining homeostasis in the body. HSP expression is induced under conditions such as oxidative stress, high temperature, and inflammation, because HSPs are needed to maintain the correct conformation of proteins. Endogenous HSP70, a subtype closely associated with the cardiovascular system, has demonstrated anti-inflammatory and protective effects during heart injury. For example, HSP70 transgenic mice are more tolerant than wild-type mice to injuries induced by activation of TLR4 with LPS. Further studies have confirmed that HSP70 significantly decreases iNOS activation and NO synthesis in cardiomyocytes [39]. When HSF1, the key molecular switch of HSP70, is knocked out in mice, thus inhibiting HSP70, and TLR4 is activated by LPS, TNF- α shows high systemic expression,

and the mortality rate increases significantly [40]. Moreover, exosomes containing HSP70 isolated from healthy participants have shown protective effects against cardiac ischemia-reperfusion injury via interaction with TLR4 [41]. In a rabbit ischemia-reperfusion model, rabbits injected with HSP70 have shown higher left ventricular ejection function, and half the myocardial cell apoptosis observed in controls [42].

These studies have indicated that HSP70 is protective against heart injury. These protective effects are associated not only with the inhibition of apoptosis [43] but also with TLR4/NF- κ B. In NF- κ B pathway activation, I κ K is required to phosphorylate I κ B, thus causing I κ B to dissociate from the p65/p50 dimer, and allowing p65/p50 to enter the nucleus and trigger the expression of inflammatory factors. HSP70 inhibits the activation of I κ K and decreases the phosphorylation of I κ B- α , thereby increasing the abundance of non-phosphorylated I κ B- α [44, 45]. Moreover, HSP70 adheres to active NF- κ B molecules, such as p65, and prevents their migration from the cytoplasm to the nucleus. HSP70 also physically blocks nuclear pores, thereby preventing translocation of the p65/p50 dimer to the nucleus [46]. In addition to its direct regulatory effects on NF- κ B, HSP70 binds TRAF6, an important intermediate in the TLR4/NF- κ B pathway [47, 48]. Therefore, HSP70 regulates the TLR4/NF- κ B pathway at multiple levels.

What Damage does TLR4/NF- κ B do to the Heart?

Endothelial Injury

The vascular endothelium is important in maintaining the stability of the cardiovascular system, and endothelial injury is closely associated with the occurrence of many cardiovascular diseases [49, 50]. Endothelial cells act as natural barriers in blood vessels by controlling the exchange of substances into and of the blood vessels; maintaining a balance between thrombosis and dissolution; and regulating angiogenesis and vascular tension. Thus, the endothelium plays important roles in the balance and stability of the internal and environments of blood vessels [49].

TLR4/NF- κ B has profound effects on the heart by affecting the function of endothelial cells. TLR4, CD14, and MD2 in endothelial cells form a trimer, which, after binding LPS, recruits the linker protein MyD88; activates downstream NF- κ B; and induces the expression of IL-1 α , TNF- β , IL-6, CXCL1, MCP-1, vascular cell adhesion molecule 1 (VCAM-1), P-selectin, and other inflammatory molecules [51]. Inflammatory cytokines recruit many neutrophils, and excessive accumulation of neutrophils can cause tissue damage. In addition, activation of the TLR4/NF- κ B pathway promotes the adhesion of monocytes to vascular endothelial cells by increasing endothelial surface expression of VCAM-1, thereby aggravating myocardial ischemia-reperfusion injury [52]. Increased activation of TLR4/NF- κ B also induces endothelial damage in the aortic valve, thus leading to calcified aortic valve disease through an inflammatory response, and resulting in heart failure in the long term. In addition to the above-mentioned effects of TLR4/NF- κ B on endothelial cells through inflammatory pathways, TLR4/NF- κ B regulates the proliferation and apoptosis of endothelial cells. Previous studies have shown that TLR4/NF- κ B pathway activation inhibits human aortic endothelial cell proliferation and mediates the apoptosis induced by oxidized low-density lipoprotein in endothelial cells [53]. To our knowledge, the MAPK pathway is the pathway most closely associated with apoptosis. However, the above studies have shown that TLR4/NF- κ B also mediates cell apoptosis. TLR4/NF- κ B directly induces the expression of apoptosis-associated genes; this effect might possibly occur because the signaling pathways are interconnected, and activation of the TLR4/NF- κ B pathway consequently affects the activation of MAPK. The association between TLR4/NF- κ B and apoptosis is interesting and will require elucidation in future research.

The damage caused by TLR4/NF- κ B to endothelial cells and consequently the heart necessitate measures to reverse these effects. Substantial experimental evidence has indicated that inhibiting the TLR4/NF- κ B signaling pathway prevents atherosclerosis [54]. Levosimendan is a clinical drug for the treatment of acute heart failure, which decreases the expression of TLR4 on endothelial cells and strongly diminishes the activity of NF- κ B by inhibiting S536 phosphorylation. Consequently,

decreases are observed in the expression of IL-6, IL-8, E-selectin, ICAM-1, and other molecules; the adhesion of neutrophils to activated endothelial cells; and LPS-induced endothelial cell death [55, 56] (Table 1).

Promotion of Cardiac Fibrosis

Myocardial fibrosis is a pathological change caused by the proliferation of cardiac fibroblasts and excessive deposition of extracellular matrix (ECM) proteins in the cardiac interstitium, and is closely associated with chronic hypoxia, myocardial injury, inflammation, and other factors. Almost all heart diseases involve myocardial fibrosis. Excessive myocardial fibrosis decreases cardiac compliance and the ejection fraction, thereby contributing to heart failure [75].

The transition from early inflammation to late fibroblast proliferation and fibrosis is the only stage at which heart tissue can be repaired [76]. During this transition, inflammatory cells release transforming growth factor beta (TGF- β). The TGF- β growth factor family is currently the most studied fibroblast growth factor family and may play a major role in myocardial fibrosis by inducing fibroblasts to transform into fibrocytes and release collagen [57]. A series of studies have shown that TLR4/NF- κ B plays an important role in myocardial fibrosis. The N-terminal C0-C1f region of cardiac myosin binding protein-C induces fibroblast activation and mediates cardiac remodeling by activating TLR4 and NF- κ B [77]. In addition, C-reactive protein, an indicator of inflammation severity, also promotes inflammation through the TLR4/NF- κ B/TGF- β pathway and causes atrial fibrosis [78].

The mechanisms underlying TLR4/NF- κ B mediated cardiac fibrosis can be summarized in three categories: mediating inflammation; promoting the proliferation and activation of fibroblasts; and enhancing the role of TGF- β . As described above, TLR4/NF- κ B is an important mediator of the immune response, whose activation results in the expression of many cytokines, such as IL-1 β and IL-6 [36]. IL-1 β stimulates the expression of proteins in the ECM, such as matrix metalloproteinases, and promotes the migration of fibroblasts, whereas IL-6 increases the proliferation of fibroblasts and causes myocardial fibrosis [59]. In addition, TLR4/

Table 1 Pathophysiological relationship between TLR4/NF- κ B and heart failure

The damage of TLR4/NF- κ B to the heart	Molecular pathophysiology	Progress in drugs	Roles of drugs	References
Endothelial injury	IL-1 β , TNF- α \uparrow VCAM-1, P-selectin \uparrow Inhibition of endothelial cell proliferation and induction of endothelial cell apoptosis	Levosimendan	Decrease in TLR4 expression on the endothelium and NF- κ B activity	[51, 53, 55, 56]
Promotion of cardiac fibrosis	TGF- β \uparrow : induction of fibroblast activation IL-1 β \uparrow : increase in ECM degradation and thus fibroblast migration IL-6 \uparrow : increase in fibroblast proliferation	Quinapril – –	Inhibition of TGF- β – –	[57, 58] [59] [59]
Cardiac hypertrophy	CaMKII, ROS, cytokines	–	–	[60–62]
Cardiomyocyte apoptosis	ROS, NLRP3 \uparrow p53 acetylation –	Pyroolidine dithiocarbamic acid Carvedilol	Inhibition of NF- κ B activation Inhibition of apoptosis Activation of K _{ATP} channels	[18, 63, 64] [64] [65–70]
Pyroptosis	NLRP3 \uparrow Caspase 1 \uparrow IL-1 β \uparrow –	Nicorandil	–	–
Autophagy	mTOR, Beclin-1, LC3-II/LC3-I	Kanglexin –	Inhibition of NLRP3 –	[71] [72–74]

NF- κ B not only increases the expression of TGF- β but also induces fibroblasts to transform into fibrocytes and release collagen [79]. TLR4/NF- κ B activation also increases the reactivity of cells to TGF- β [80], thus further enhancing the effects of TGF- β on myocardial fibrosis (Table 1).

Cardiac Hypertrophy

Cardiac hypertrophy occurs because the heart is stimulated by adverse factors – such as increased heart load caused by hypertension and valvular disease, myocardial infarction, or inflammation – for prolonged time periods. Long-term adverse stimulation leads to compensatory changes in the heart to counter these effects, but excessive hypertrophy causes heart failure, which is fatal when untreated [81].

TLR4 and NF- κ B are closely associated with myocardial hypertrophy [82, 83]. Here, we describe the effects of TLR4/NF- κ B mainly in terms of induction of inflammation and oxidative stress, as well as regulation of the sympathetic nervous system and blood pressure. LPS activates TLR4 and Ca²⁺ calmodulin-dependent protein kinase II (CaMKII) in cardiomyocytes [60]. CaMKII is a signaling molecule that is activated by ROS and subsequently induces inflammatory molecule expression and causes adverse consequences. Excessive CaMKII expression results in myocardial hypertrophy and heart failure [61, 62]. However, when MyD88, a molecule downstream of TLR4, is knocked out, CaMKII expression decreases, and consequently diminishes inflammation and myocardial hypertrophy [60]. Importantly, the failure of other organs can also trigger heart failure, TLR4/NF- κ B is involved in this process; for example, under conditions of renal ischemia-reperfusion, a systemic inflammatory response is induced, and TLR4/MyD88/NF- κ B mediates cardiac hypertrophy [84]. Many studies have shown that knocking down the expression of TLR4 or NF- κ B at the gene or protein level significantly alleviates myocardial hypertrophy caused by various factors, mainly through decreasing the expression of inflammatory cytokines and ROS [85–89]. More importantly, TLR4/NF- κ B is closely associated with the regulation of blood pressure. Studies have shown that subcutaneous infusion of angiotensin II

(Ang II) increases the levels of TLR4 in an Ang II-induced hypertensive rat model, thereby inducing myocardial inflammation and increasing cardiac sympathetic nerve activity; elevated blood pressure subsequently increases cardiac afterload and hinders left ventricular contraction, thereby eventually leading to ventricular hypertrophy [90, 91]. Blocking TLR4/NF- κ B in the brain in a spontaneously hypertensive rat model significantly decreases blood pressure [92]. Investigation of the underlying mechanisms has indicated that inhibiting TLR4/NF- κ B decreases the body's responsiveness to Ang II and downregulates norepinephrine levels in the circulation [90] (Table 1).

Cardiomyocyte Apoptosis

Programmed cell death, under physiological conditions, is necessary for the body to remove abnormal cells [93]. However, under conditions such as trauma or stress, excessive apoptosis can cause organ failure, including heart failure [94].

Inflammation and oxidative stress induced by TLR4/NF- κ B are closely associated with apoptosis. Ischemia-reperfusion injury induces myocardial production of large amounts of ROS, which cause myocardial damage [95] and also activate apoptotic signals resulting in cell death [96]. In addition, the TLR4/NF- κ B pathway activates NLRP3 inflammasomes and mediates LPS-induced apoptosis [63]. The activation of apoptosis by TLR4/NF- κ B is associated with increased acetylation of p53, which enters the nucleus and promotes the transcription of apoptosis genes [18]. Blocking these pathways pharmacologically, with an antibody inhibiting TLR4 or pyrrolidine dithiocarbamic acid, further inhibits NF- κ B activation and significantly decreases the apoptotic index (Bcl-2/BAX) of cardiomyocytes [64]. Carvedilol can also be used to protect the heart by inhibiting apoptosis in myocardial cells [64]. These results demonstrate that TLR4/NF- κ B is closely associated with cardiomyocyte apoptosis and causes heart failure partly via the apoptotic pathway (Table 1).

Pyroptosis

Pyroptosis is a type of programmed cell death closely associated with inflammation. After

stimulation by specific signals, cells begin to swell and undergo cell membrane rupture, thereby releasing inflammatory cytokines, which further aggravate inflammation [97]. Pyroptosis is closely associated with disease; for example, lung injury caused by endotoxemia is partially mediated by endothelial pyroptosis [98], and nicotine, a harmful substance in cigarettes, promotes atherosclerosis through endothelial pyroptosis [99]. In addition, oxidative stress, hyperglycemia, dyslipidemia, and inflammation all trigger focal death, which in turn can mediate atherosclerosis, ischemic cardiomyopathy, diabetic cardiomyopathy, and myocardial hypertrophy, and ultimately lead to heart failure [100].

TLR4/NF- κ B plays an important role in triggering focal death, a cell death mechanism closely associated with inflammation. Pathogens stimulate PRRs, such as TLR4, thus activating downstream NF- κ B, promoting the formation of NLRP3 inflammasomes and the activation of caspase-1, and leading to focal death [65, 66]. In a mouse model of diabetic cardiomyopathy, hyperlipidemia has been found to increase the expression of TLR4/NF- κ B in the aorta and to upregulate NLRP3, caspase-1, and IL-1 β , thereby leading to the death of vascular endothelial cells [67–69]. During the activation of this pathway, NLRP3 activation is also closely associated with thioredoxin (TRX) interacting protein (TXNIP). TXNIP binds TRX under normal conditions. However, under oxidative stress conditions, ROS promote the dissociation of TXNIP from TRX, and the free TXNIP directly interacts with NLRP3 and subsequently mediates downstream inflammation and pyroptosis [101]. Free TRX also has an antioxidant role as a reducing substance. Thus, the two proteins result in a delicate balance between oxidation and reduction through NLRP3 [102].

Excessive pyroptosis is detrimental to the heart, and measures should be taken to prevent its occurrence. Nicorandil, a medicine commonly used for clinically treating angina pectoris, partially inhibits pyroptosis via the TLR4/MyD88/NF- κ B/NLRP3 signaling pathway, possibly through the activation of ATP-dependent K⁺ ion channels [70]. The anthraquinone compound kanglexin also protects against myocardial ischemic injury in mice by inhibiting NLRP3 and pyroptosis [71] (Table 1).

Autophagy

Autophagy is a process in which intracellular lysosomes degrade damaged proteins and organelles. Normal autophagy is necessary for maintaining cell homeostasis and for organelle renewal, but overactive autophagy may lead to organ damage [103, 104]. Previous studies have shown that autophagy mediates not only epithelial damage in airway inflammation [105] but also myocardial damage [106]. In addition, neuronal apoptosis mediated by TLR4/NF- κ B activation is associated with traumatic brain injury in rats [107].

A relationship exists between TLR4/NF- κ B and autophagy. In myocardial injury caused by sepsis, TLR4/NF- κ B increases the autophagy in myocardial cells, thereby damaging them [72]. When a specific inhibitor is used to inhibit TLR4 and NF- κ B activity, myocardial injury markers, such as creatine kinase and lactate dehydrogenase, decline, and inflammatory cytokines, such as IL-6 and TNF- α , are downregulated. More importantly, Beclin-1 protein levels decrease, as does the ratio of LC3-II/LC3-I – proteins directly associated with autophagy [73]. TLR4 knockout also regulates mTOR activity [74]. The above results suggest that TLR4/NF- κ B and apoptosis may be related through mTOR, Beclin-1, and LC3-II/LC3-I (Table 1).

Relationships between TLR4/NF- κ B and Major Diseases Causing Heart Failure

Hypertension

Hypertension is an important cause of heart failure, because it requires the heart to maintain a high load operation state for a long time, thus eventually deteriorating cardiac function and resulting in heart failure [108]. Most hypertension cases are primary hypertension with no clear etiology. However, some studies have shown that hypertension is closely associated with inflammation and oxidative stress, and TLR4/NF- κ B plays an important role in the occurrence of hypertension.

TLR4 expression is elevated in the hypothalamic paraventricular nucleus (PVN) in a hypertensive rat model [92]. The renin-angiotensin system (RAS) is an important blood pressure regulation system,

in which TLR4 plays an extensive role. First, TLR4 mediates the hypertension induced by AngII. When TLR4 is blocked in the brain, the blood pressure-raising effect of AngII substantially weakens [90]. TLR4 also affects the production of Ang II. Resistin, a hormone that regulates vascular function [109], acts together with TLR4 in promoting the expression of angiotensinogen, the precursor of AngII. When the TLR4 gene is specifically knocked out, the effect of resistin on promoting the expression of angiotensinogen disappears [110]. Thus, TLR4 plays an important role in regulating RAS.

In addition to regulation of the RAS, TLR4/NF- κ B is also broadly involved in inflammation. Inflammation has been reported to be the main factor contributing to the progression of hypertension-induced cardiac remodeling [111]. TLR4/NF- κ B-mediated inflammatory stimulation induces the secretion of TNF- α and IL-1 β , which facilitate cardiovascular disease occurrence and development [90]. In a hypertensive rat model, TLR4/NF- κ B knockdown in the PVN decreases the activity of TNF- α , IL-1 β and iNOS, thus lowering blood pressure and ameliorating cardiac hypertrophy [92].

Therefore, TLR4/NF- κ B plays a crucial role in the occurrence and development of hypertension. Some studies have also shown that blocking TLR4/NF- κ B has substantial effects in controlling hypertension. For example, statins not only effectively control blood lipids, but also decrease the expression of TLR4 on immune cells and inhibit NF- κ B activity, thereby decreasing the inflammatory response of monocytes to LPS and the secretion of inflammatory cytokines [112, 113]. PTCd, an inhibitor of NF- κ B, inhibits the activation of NF- κ B, decreases the expression of TLR4 and AngII, and effectively reverses left ventricular remodeling and slows the development of heart failure [114].

Myocardial Infarction

Myocardial infarction is another important cause of heart failure. Myocardial cells die after ischemia and hypoxia, and are not renewable, thus resulting in a loss of ejection function [115]. The expression of TLR4 and downstream NF- κ B in myocardial infarction tissues is significantly elevated, thus strongly suggesting that TLR4/NF- κ B is associated with myocardial infarction [116]. In fact, the role

of TLR4/NF- κ B in the development of myocardial infarction runs through the whole process. Inhibition of the TLR4 pathway before myocardial infarction has been found to result in a significantly smaller myocardial infarct size than no intervention in wild-type mice subjected to ischemia and hypoxia [117, 70]. A similar phenomenon has been observed in TLR4 mutant mice [118]. Moreover, TLR4/NF- κ B plays an important regulatory role in cardiac function after myocardial infarction. Malignant arrhythmia easily occurs after myocardial infarction, and its pathogenesis is associated with TLR4/NF- κ B-mediated inflammation and sympathetic hyperfunction, thus substantially decreasing the cardiac ejection function. However, after TLR4/NF- κ B inhibition, the incidence of malignant arrhythmia after myocardial infarction significantly decreases [119, 20]. Research has demonstrated that the main mechanisms through which TLR4/NF- κ B aggravates myocardial infarction include inflammation [35], oxidative stress [120], and pyroptosis [70], and apoptosis [121].

Therefore, inhibition of TLR4/NF- κ B is important for alleviating myocardial infarction and thus heart failure. Nicorandil, which is commonly used in clinical practice to improve the prognosis of cardiovascular events, has been demonstrated to decrease pyroptosis in myocardial infarction by inhibiting the TLR4/MyD88/NF- κ B/NLRP3 pathway [70]. In addition, perindopril, beyond inhibiting cardiac remodeling, inhibits cardiomyocyte apoptosis in a mouse model of acute myocardial infarction, through the TLR4/NF- κ B pathway, thus playing an important role in alleviating heart failure [121].

Viral Myocarditis

Viral myocarditis results from viral infection of the heart and induces localized or widespread inflammation of the myocardial tissue and arrhythmia; it has been identified as a major cause of dilated cardiomyopathy, which in turn can lead to heart failure. TLR4/NF- κ B plays an important role in the inflammatory response and fibrosis of coxsackievirus B3-induced viral myocarditis [122]. In addition to causing inflammation, TLR4/NF- κ B can affect viral replication: compared with wild-type mice, TLR4-deficient mice not only show less coxsackievirus B3 replication but also have stronger resistance to the virus

[123]. Interestingly, the TLR4-MyD88-NF-κB pathway and the TLR4-TRIF-IRF3 pathway have opposite effects in viral myocarditis. The TLR4-MyD88-NF-κB pathway exacerbates viral myocarditis [124],

whereas TLR4-TRIF-IRF3 effectively protects against viruses. Inhibition of TLR4-TRIF-IRF3 leads to cardiac remodeling and heart failure due to severe viral infection [125]. Although the two pathways

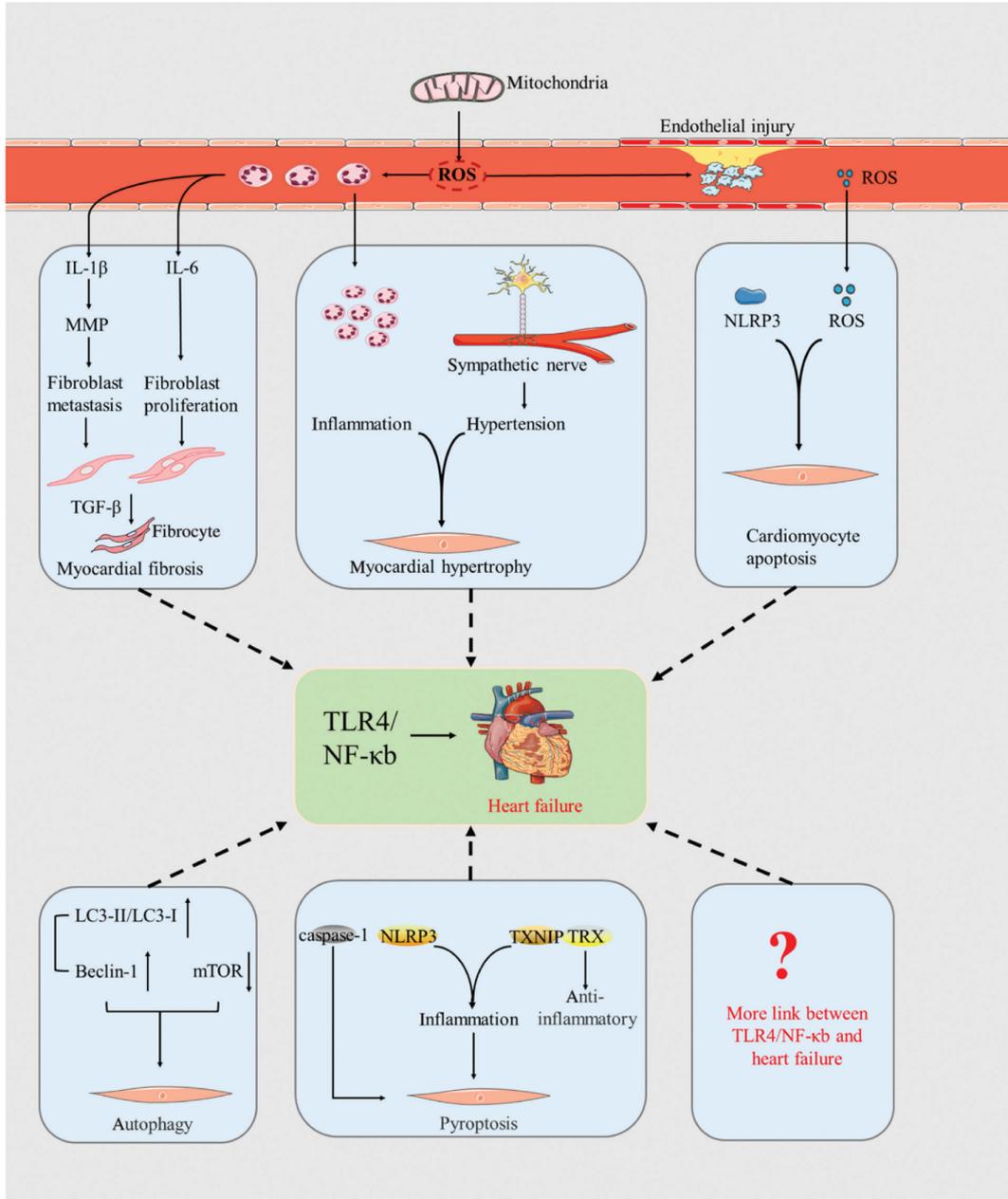


Figure 2 Overactivated TLR4/NF-κB contributes to heart failure through multiple pathways. ROS produced by the mitochondrial electron transport chain and inflammatory cells themselves can damage the heart and vascular endothelia, thus leading to thrombosis and further aggravation of myocardial hypoxia. Simultaneously, inflammatory cells secrete IL-1β and IL-6. IL-1β induces the expression of MMP in the extracellular matrix, thus promoting the transfer of fibroblasts. IL-6 induces the proliferation of fibroblasts, which differentiate into fiber cells under the action of TGF-β, thus leading to myocardial fibrosis. The increase in inflammation and sympathetic nerve activity aggravates myocardial hypertrophy. Excessive production of NLRP3 and ROS induces cardiomyocyte apoptosis. In addition, the changes in LC3-II/LC3-I, Beclin-1, mTOR, and other molecules promote autophagy. TXNIP, after dissociating from the TXNIP/TRX complex, interacts with NLRP3 and induces pyroptosis. The above phenomena are closely associated with TLR4/NF-κB, but represent only part of the pathway through which TLR4/NF-κB causes heart failure. More molecular connections remain to be explored.

mentioned above are TLR4 mediated, they have opposite effects, because TLR4-MyD88-NF- κ B is responsible mainly for triggering the inflammatory response and excessive inflammation, thereby damaging myocardial tissue and causing arrhythmia. However, the activation of the TLR4-TRIF-IRF3 pathway is responsible mainly for the synthesis of IFN, a powerful anti-viral factor [126].

Summary and Future Prospects

TLR4 and downstream NF- κ B signaling are important for the normal function of cells. Previous studies have emphasized that NF- κ B plays a protective role in the heart under stress conditions, such as ischemic myocardial injury or cardiac hypoxia [127]. However, extensive evidence has also indicated that overactive TLR4/NF- κ B plays an important role in the occurrence and development of heart failure, through mechanisms mainly including (i) oxidative stress, (ii) inflammation, (iii) myocardial fibrosis, (iv) endothelial damage, (v) myocardial hypertrophy, and (vi) apoptosis. TLR4/NF- κ B also plays an important role in the heart as well as other organs, such as the kidneys [128], nervous system [129], digestive system [130], and lungs [131]. The mechanism underlying these effects is associated with the production of inflammatory cytokines and ROS. ROS production is the main pathway through which neutrophils exert antibacterial effects. Cytokines such as TNF- α are also important inflammatory mediators against bacteria. Thus, excessive removal of these compounds may increase the likelihood of infection [132, 133]. However, if produced in excess, these compounds can damage the body, and appropriate anti-inflammatory substances may be required to eliminate them. Most importantly, drugs that specifically antagonize TLR4/NF- κ B may be used to delay the progression of heart failure. Clinically, some drugs used for the treatment of cardiovascular diseases, such as levosimendan [134], carvedilol [135], and nicorandil [70], exhibit TLR4 antagonism. However, not all molecules that antagonize TLR4 can be applied in clinical treatment, because most of them fail to demonstrate effectiveness from preclinical to clinical stages. Such drugs include Eritoran and TAK-242. Eritoran is a specific TLR4 antagonist that has been found to significantly decrease the infarct size of the heart

in a mouse model of ischemia-reperfusion injury [136]. Another TLR4-specific antagonist, TAK-242, alleviates damage to the heart by inhibiting monocyte-mediated inflammation in a mouse ischemia-reperfusion model, thereby diminishing the loss of cardiac ejection function [137]. These two drugs, despite their potential, have not been studied in large animal models but have been tested in patients. Unfortunately, trials of both drugs did not reach hard clinical endpoints [138, 139]. Beyond the above-mentioned factors causing heart failure, some recent reports have shown that the intestinal microbiome also affects heart function through its metabolites [140, 141]. Our team has long been committed to heart failure research, and we have recently found that regulating disorders in the intestinal flora can effectively protect against heart failure [142], thus not only confirming that the body must be considered holistically but also providing a broader target for the treatment of heart failure. However, reports on whether the association between the intestinal microbiome and the heart is also mediated by TLR4/NF- κ B are lacking; examining this possibility is a goal of our future research (Figure 2).

Abbreviations

NF- κ B:	nuclear factor kappa-B
LPS:	lipopolysaccharide
ROS:	reactive oxygen species
TLR:	Toll-like receptor
MyD88:	myeloid differentiation factor 88
TRIF:	Toll/Interleukin-1 receptor domain-containing adaptor-inducing interferon- β
IRAK4:	IL-1R-associated kinase 4
TRAF6:	tumor necrosis factor receptor-associated factor 6
I κ B:	inhibitory kappa B
I κ K:	inhibitory kappa B kinase
TNF:	tumor necrosis factor
NOS:	nitric oxide synthase
NLRP3:	NLR family pyrin domain containing 3
HSPs:	heat shock proteins
VCAM-1:	vascular cell adhesion molecule 1
ECM:	extracellular matrix
TGF- β :	transforming growth factor beta
CaMKII:	Ca ²⁺ calmodulin-dependent protein kinase II

TRX: thioredoxin
 TXNIP: thioredoxin interacting protein
 PVN: paraventricular nucleus
 RAS: renin-angiotensin system

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Conflicts of Interest

No competing financial interests exist regarding this work.

Author Contributions

JD Z contributed to the writing of the manuscript; H L, SS M, HL Z, and JJ H contributed to the production of illustrations; and JF C and HY G contributed to the design of the project.

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