Published in final edited form as:
Cardiovasc Ther. 2009 ; 27(2): 117–123. doi:10.1111/j.1755-5922.2009.00077.x.

Innate Immunity and Toll-like Receptor Antagonists: A Potential Role in the Treatment of Cardiovascular Diseases

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Abstract

Toll-like receptors (TLRs) are germline-encoded receptors that recognize various pathogen-associated molecular patterns (PAMPs). They are key components of the innate immunity which are activated in response to pathogens as well as non-pathogenic components of damaged tissues. TLR agonists have been developed to treat allergies, cancers, and chronic infections by upregulating the innate immune system. TLR antagonists may be used to treat a number of inflammatory conditions, such as rheumatoid arthritis and systemic lupus erythematosus. Recent research also has shown that TLRs are involved in the pathogenesis of atherosclerosis, thrombosis, myocardial remodeling, ischemic/reperfusion injury, and valvular disease. This article reviews the current experimental and clinical evidence for the role of TLRs in the cardiovascular system, and examines the mechanisms by which TLR antagonists could potentially be used in targeted therapy.

Keywords

Atherosclerosis; Cardiovascular disease; Myocardial Ischemic/reperfusion injury; Thrombosis; TLR antagonist; TLRs; Valvular disease

Introduction

The innate immune system has long been regarded as the first line of defense against foreign pathogens. However, more recently it is recognized that the involvement of nonpathogenic components of necrotic or damaged tissues can activate innate immunity. Thus, rather than simply responding to “foreign” material, the innate immune system responds to “danger” signals that can be either microbial or endogenous in origin [1]. Toll-like receptors (TLRs) are germline-encoded receptors that recognize an array of pathogen-associated molecular patterns. TLRs induce a rapid innate immune response and serve as a bridge to long-term adaptive immune responses. There are 10 human and 13 murine TLRs characterized to date. Each recognizes different ligands that produce distinctive responses depending on the context, such as the nature of the “danger” signal and the specific cell type activated. The last decade has seen major advances in the field of TLR research. As further insight is gained, various drugs have been developed to target individual receptors for the treatment of specific diseases. Most of the TLR drugs marketed today or that are in development are agonists. The rationale behind TLR agonist development centers on the activation of effector cells of the innate immune system in order to trigger the adaptive immune response against specific targets, such as viruses

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Conflict of Interest: The authors have no conflict of interest.

and cancer cells. Numerous clinical trials have been launched to study the efficacy and safety profile of TLR agonists directed at treating allergies, cancer, and chronic infections such as HCV and HIV.

Investigations of TLR agonists have also drawn interest into the prospective use of TLR antagonists to decrease inflammatory responses mediated by the innate immune system. For example, TLR4 antagonists Eritoran and Tak242 were developed for the treatment of severe sepsis. In addition to infectious agents, TLRs have also been shown to respond to a variety of endogenous ligands derived from substances released as a result of cell death or tissue injury [2]. Thus, other research efforts are under way to study the role of TLR signaling in the pathogenesis of noninfectious conditions such as lupus, rheumatoid arthritis, inflammatory bowel disease, and interestingly, cardiovascular diseases.

In addition to the classic immune cells such as macrophages and monocytes, other non-bone marrow-derived cells were also shown to actively participate in the inflammatory cascade through TLR signaling. TLRs are expressed in almost all cells of the heart—TLR2, 3, 4, and 6 are found in cardiomyocytes, and TLR1 through 6 are found in the smooth muscle and endothelial cells of the vasculature [3]. Therefore, it is not surprising that TLRs are involved in the development of atherosclerosis, thrombosis, myocardial remodeling, ischemic/reperfusion injury, and even valvular disease. Here we review the current experimental and clinical evidence for the role of TLRs in the cardiovascular system and examine the mechanisms by which TLR antagonists could potentially be used in targeted therapy.

Atherosclerosis and Coronary Artery Disease

Atherosclerosis is a chronic inflammatory disease of the arterial vasculature, largely due to the interplay between modified lipoproteins and cytokines, which eventually results in plaque initiation and progression (Figure 1). There is evidence that TLRs, notably TLR2 and TLR4, are involved in the development of atherosclerotic disease.

TLR2 expression was recently shown to be increased in endothelial cells in the regions with disturbed blood flow in atherosclerosis-prone $LDLr^{-/-}$ mice. Furthermore, diet-induced hyperlipidemia increased TLR2 expression at these sites [4]. TLR2 has also been shown to be critical in the progression of atherosclerosis in mouse models. Inactivation of TLR2 leads to a reduction in lipid accumulation in $ApoE^{-/-}$ mice as well as a reduction in chemokine production, specifically MCP-1 levels. These findings suggest that TLR2 is important in atherosclerosis independent of dietary cholesterols [5].

TLR4 also plays an important role in atherosclerotic development and progression. TLR4 is expressed by macrophages in both human atherosclerotic lesions and at the site of plaque rupture in patients with acute myocardial infarction [6]. It has been established that oxidized LDL and its components serve as endogenous TLR4 ligands [7]. Levels of endogenous TLR ligands, EDA and heat shock protein 60 (hsp60), and the mRNA encoding TLR2 and TLR4 are increased in $ApoE^{-/-}$ mice with advanced atherosclerosis [8]. TLR4 protein expression was also shown to be upregulated in vascular smooth muscle cells (VSMCs) of atherosclerotic arteries [9]. Signaling through TLR4 in VSMCs resulted in a proinflammatory phenotype and increased LDL and extracellular matrix deposition, which contributes to the acceleration of atherosclerotic lesions [10].

Studies have also shown that exogenous ligands, such as lipopolysaccharide (LPS) in sepsis or components of *Porphyromonas gingivalis* in periodontal infections, accelerate atherosclerosis by engaging TLRs. In addition, IFN-α, produced by plasmacytoid dendritic cells in atherosclerotic plaques, was found to enhance TLR4 signaling by sensitizing these cells to the TLR4 ligand, LPS. IFN-α upregulation of TLR4 expression increases the production of

TNF-α, IL-12, and MMP-9, some of the key players in plaque destabilization [11]. These data suggest that TLRs may be the link between inflammation and atherosclerotic disease, in the presence or absence of infection [12].

It is unclear whether TLR2 and TLR4 independently or cooperatively contribute to atherosclerosis. There is additional evidence to suggest that there may be synergy between the two receptors in the progression of atherosclerosis. Transfection of cDNA encoding human TLR2 and TLR4 in the carotid arterial walls of rabbits accelerates atherosclerosis, while a less significant effect is seen with the transfection of either receptor individually [13].

The evidence accumulated by basic research suggests that TLRs play a critical role in atherosclerosis and coronary artery disease, which has led many groups to examine their clinical significance. One group has found that TLR2 and TLR4 expressions correlated with the extent and severity of coronary artery disease in patients with stable angina [14].

Thrombosis

Platelets are key components of thrombosis, a process in which vessel damage results in clot formation, eventually leading to ischemia of the damaged area (Figure 1). Recent studies have shown that human platelets express TLRs [15,16]. The functionality of TLRs in platelets was demonstrated in several studies focused on the stimulation of TLR4 by LPS, thereby inducing neutrophil sequestration and thrombocytopenia. In addition, TLR4 stimulation causes the priming of platelet aggregation activated by epinephrine, ADP, and arachidonic acid [17,18]. Besides TLR4, TLR2 is also a functional receptor in platelets [19]. Treatment with PAM_3CSK4, a synthetic ligand of the TLR2/TLR1 heterodimer, directly resulted in platelet aggregation, adhesion, granular secretion, interaction with leukocytes, and reactive oxygen species (ROS) production. These effects were mediated by PI3-K/Akt signaling [19].

Based on these findings, it is likely that the activation of the innate immune system, either by pathogens or by tissue injury, can lead to thrombosis and subsequent coronary events. These mechanisms could explain the relationship between inflammation and thrombosis, as described in several studies [20,21]. Further studies are needed to investigate the clinical significance of TLR upregulation in thrombosis and myocardial ischemia.

Congestive Heart Failure and Myocardial Remodeling

Congestive heart failure occurs in many pathological conditions including coronary artery disease, valvular disease, and hypertension. Particularly following myocardial infarction, a compensatory but maladaptive remodeling occurs in order to maintain cardiac output (Figure 1). However, this remodeling process leads to diastolic impairment and further decline of cardiac function.

There is evidence that TLRs are involved in the pathogenesis of heart failure and myocardial remodeling. Studies have shown that TLR2, 3, 4, 5, 7, and 9 are expressed on cardiac myocytes. TLR2, 4, and 5 signaling results in a robust inflammatory response via NFκB, as well as decreased contractility both in human myocardium and in murine cardiomyocyte cell lines [22,23].

Among the TLR family, TLR4 is the most extensively studied in the pathogenesis of cardiomyopathy. TLR4 activation not only triggers an inflammatory response but also results in extracellular matrix degradation, an important step in left ventricular (LV) remodeling. On the other hand, TLR4 deficiency leads to a reduction in LV remodeling and a preservation of systolic function post-myocardial infarction (MI) in mice [24]. In addition, studies using $TLR4^{-/-}$ mice demonstrated a concomitant decrease in protein levels of TLR4 adaptor MyD88

and an increase in antihypertrophic JNK signaling, resulting in a significantly improved survival post-MI [25]. Another study investigating the prognostic value of activated monocytic TLR4 after acute myocardial infarction found that activated TLR4 was an independent predictor of 30-day major adverse clinical outcomes, including advanced Killip score, overt congestive heart failure, New York Heart Association (NYHA) class ≥2, or death [26].

Interestingly, one recent study also implicates TLR9 in the reduction of myocardial contractility by showing the inhibitory effects of CpG-ODN, a synthetic exogenous TLR9 ligand, on sarcomeric shortening. In addition, TLR9 activation in cardiomyocytes leads to a strong inflammatory response marked by the release of TNF-α, IL-1β, IL-6, and activation of NFκB and iNOS [27].

Myocardial Ischemic/Reperfusion Injury

Myocardial ischemic/reperfusion (MI/R) injury is caused by the restoration of blood flow to the heart following an ischemic event. Ironically, this reperfusion process leads to an inflammatory response that causes further damage to viable tissue around the infarct, likely through accelerated apoptosis (Figure 1). TLRs are implicated in MI/R injury, suggested by the fact that the use of a TLR4 antagonist Eritoran in mice protects against this injurious process [28]. This protective mechanism may be attributed to attenuated inflammation, such as decreased myeloperoxidase activity, leading to smaller infarcts compared to controls [29]. Further studies have suggested that extracellular heat shock cognate protein 70 (HSC70), released by the myocardium during MI/R injury, plays a key role in the postischemic inflammatory response via TLR-4 signaling [30]. TLR2 also plays a role in MI/R injury, likely related to its modulation of leukocyte activity, which mediates coronary endothelial dysfunction [31].

Valvular Heart Disease

Few studies have investigated the role of the innate immunity on valvular disease, which often involves calcification (Figure 1). Recent research has shown that human aortic valve interstitial cells express TLR2 and TLR4. Furthermore, TLR2 and TLR4 upregulation results in an increase in gene expression of osteogenic factors, such as BMP-2 and Runx2, likely contributing to the pathogenesis of aortic stenosis [32,33]. A small-scale study showed that plasma and tissue TNF-α and IL-6 levels correlated with the amount of calcium deposits in human aortic valve stenosis, which may be a result of upregulated TLR2 and TLR4 [34]. More studies are needed to examine the role of the innate immunity and TLRs in valvular heart disease.

Potential Uses of TLR Antagonists

As more data emerge supporting the role of TLRs in various cardiovascular diseases, there is a growing interest in therapeutics targeting TLRs and components of the downstream proinflammatory signaling cascade.

Since TLRs contribute significantly to the pathogenesis of atherosclerosis and other cardiovascular diseases, researchers have been prompted to study the effects of available anti-inflammatory cardiovascular drugs on TLR activity. For instance, statins have been shown to inhibit the TLR4-mediated inflammatory response in certain individuals with a specific TLR4 genotype, explaining the added benefit of statins on the cardiovascular risk of a specific subset of the population [35]. One study showed that fluvastatin negatively regulates monocyte TLR4 signaling in patients with congestive heart failure, suggesting a possible beneficial effect of statins on cardiac remodeling [36]. In addition, endothelial lipase was shown to be upregulated by LPS through TLR4, which leads to the uptake of LDL by macrophages. This increase was

shown to be blocked by simvastatin [37]. Thus, statins could provide an additional level of cardioprotection by modulating TLR activity, secondary to its well-established effects on hyperlipidemia.

Angiotensin receptor blockers (ARBs) have been shown to have TLR antagonist activity, a study based on the rationale that angiotensin II is involved in the vascular inflammatory response [38]. Stimulation with TNF-α and angiotensin II increased TLR4 mRNA levels in cultured human VSMCs [9]. Candesartan inhibits PAM_3CSK4 and LPS-induced TLR2 and TLR4 mRNA and protein expression in human monocytes *in vitro* [39]. Thus, ARBs, in addition to their antihypertensive and cardiac remodeling effects, have potential added benefits in treating other types of cardiovascular diseases by modulating TLR-mediated inflammatory response.

Although some currently marketed drugs have shown to have TLR antagonist activity, targeted TLR2 and TLR4 antagonists may prove to be more effective. Drugs can be developed to target several different steps in TLR2 and TLR4 signaling: (1) interaction between the ligand and receptor; (2) interaction between the receptor and adaptors of the signaling pathway; and (3) enzymatic activity of downstream factors. Blocking of the ligand–receptor interaction can be done either by using a neutralizing antibody, soluble decoy receptors, or a mimetic ligand. For example, synthetic derivatives of LPS lipid A from *P. gingivalis* were found to be potent antagonists of human TLR4, as shown by Zhang et al. [40]. Soluble forms of human TLR2 (sTLR2) have been shown to be released by monocytes, and the depletion of sTLR2 resulted in an exaggerated inflammatory response [41]. Patients with post-MI heart failure have been shown to have markedly decreased sTLR2 compared to controls [42]. Anti-TLR4 neutralizing antibodies were also found in many studies to suppress NFκB activity, making it another potential for drug development [43]. Thus, development of synthetic, soluble TLRs may be an effective way to block TLR signaling.

Downstream targets of TLR signaling are also candidates for drug design. Adaptors such as MyD88 and Mal, as well as kinases like IRAK, p38, and JNK, could be antagonized to attenuate TLR-mediated inflammation.

The therapeutic effect of two TLR4 antagonists, including E5564 (Eritoran) by Eisai, Inc., and TAK-242 by Takeda Pharmaceutical Company, are currently undergoing phase III clinical trials, mainly for the treatment of severe sepsis. Eisai announced in 2005 that phase II trials for Eritoran showed a 12% reduction in the mortality rate in septic patients in the high-dose treatment group compared to placebo [44]. The drug was largely well-tolerated, although self-limited phlebitis was noted in 6.7% of the patients. For cardiovascular diseases, Eritoran also seemed to have some preclinical benefits. As previously mentioned, it was shown to attenuate myocardial I/R injury by inhibiting TLR4 [28]. Thus, more experiments are warranted to study the therapeutic and side effects of Eritoran and TAK-242 on other TLR4-mediated cardiovascular diseases. Currently, there are no additionally published clinical trial data looking at TLR antagonists as a therapeutic for cardiovascular diseases.

Conclusions

There is growing evidence implicating TLR2 and TLR4 in the pathogenesis of atherosclerosis, thrombosis, congestive heart failure/myocardial remodeling, ischemic/reperfusion injury, and valvular disease (Figure 1). Antagonism of these TLRs may modulate the inflammatory response and potentially lessen the deleterious processes that lead to the pathogenesis of certain cardiovascular diseases. More studies are necessary to examine the use of these drugs in specific cardiovascular diseases, along with long-term evaluation of the effects of TLR antagonists.

Acknowledgments

We would like to thank Hannah Iafrati for her assistance with the illustration.

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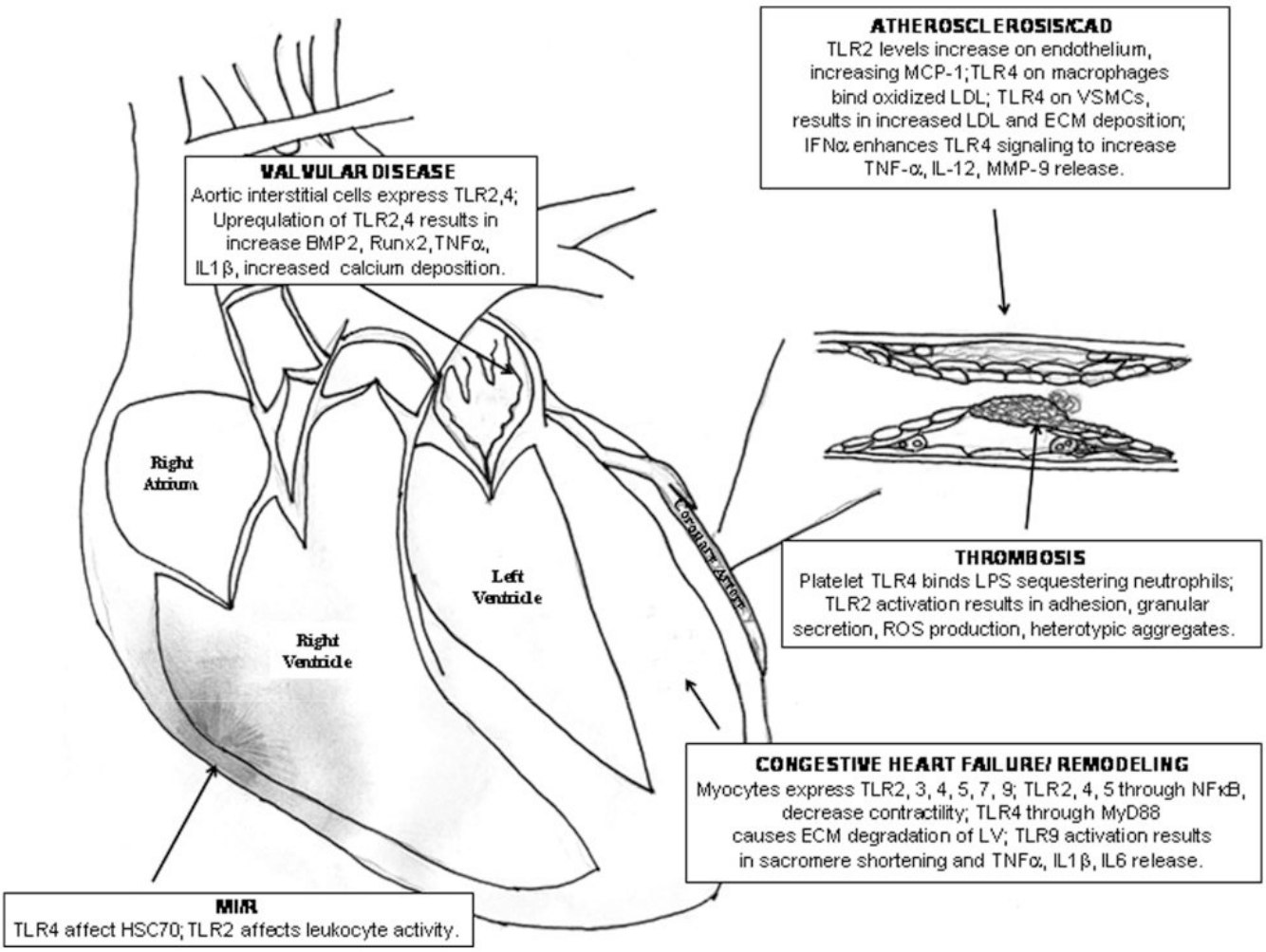

Figure 1.
Schematic summarizing the role of TLRs in cardiovascular disease.