1. ABSTRACT

Inflammatory process plays a fundamental role in ischemic coronary artery disease (CAD) in terms of both the etiology of atherosclerosis and the pathophysiology of CAD. In particular, chronic inflammation plays a key role in coronary artery plaque instability and subsequent occlusive thrombosis. It is therefore important to clarify the mechanism underlying the activation of the immune response in the pathogenesis of CAD. Currently 10 toll-like receptors (TLRs) have been reported in mammalian species, and these appear to recognize distinct pathogen-associated molecular patterns controlling innate immune responses. In recent studies, signaling of two forms of human TLR (TLR2 and TLR4) has been shown to be involved in the pathogenesis of CAD, establishing a key link between the progression of coronary atherosclerosis and immune response to both foreign pathogens and endogenously generated inflammatory ligands. A better understanding of TLR signal may provide a novel therapeutic agent for the treatment of CAD. This review summarizes the relationship between the pathogenesis of ischemic coronary artery disease and the human TLR system.

2. INTRODUCTION

It has become evident that atherosclerosis is a chronic inflammatory disease involving an immune response during its initiation and progression (1, 2). Clinical and histopathological reports suggest that a chronic inflammatory process plays a key role in coronary artery plaque instability and subsequent occlusive thrombosis (3, 4). In an autopsy study, ruptured and vulnerable plaques from patients who died of acute myocardial infarction (AMI) showed greater inflammation of macrophages and lymphocytes than stable plaques from patients with stable angina, which suggests that activation of the immune system is involved in the progression of ruptured and vulnerable plaques (5). This report has also suggested that inflammatory infiltration of macrophages and lymphocytes into coronary plaque may be related to acute and fatal coronary events (5). It is therefore important to clarify the mechanism underlying activation of the immune system in the pathogenesis of ischemic heart disease.

A retrospective analysis has revealed that baseline white blood cell count is an independent predictor of mortality in left ventricular dysfunction, specifically in
Role of Toll like receptor signaling pathway in CAD

subjects with ischemic heart disease (6). Several studies have demonstrated that peripheral monocyteosis and leukocytosis are associated with the occurrence of adverse cardiac events, such as cardiac death, recurrent MI and heart failure, in patients with AMI (7, 8). It has also been reported that peripheral monocyteosis is associated with left ventricular dysfunction and dilatation after AMI, suggesting that monocyteosis may possibly play a role in the development of ventricular remodeling (8). Meisel et al. found a correlation between peripheral monocyteosis and myocardial infarct size (9). Our previous study showed that peripheral monocyte counts increased in AMI patients with heart failure compared to those without heart failure (10). These observations therefore suggest that the activation of a monocyte-related inflammatory reaction may both reflect and potentially drive cardiac events after AMI. It has been reported that levels of monocyte-related cytokines, notably tumor necrosis factor (TNF)-α and interleukin (IL)-6, are raised in peripheral blood mononuclear cells (PBMC), including monocytes / macrophages and leukocytes, in patients with AMI (11, 12). These monocyte-related cytokines may contribute to the inflammatory and subsequent immune responses after AMI. An immune system which consists of monocyteosis and subsequent production of inflammatory cytokines is related to the activation of a systemic inflammatory reaction, and is involved in the occurrence of adverse cardiac events due to ventricular dysfunction and remodeling after AMI. From these observations, it follows that the immune system consisting of monocyte-related cytokine production may play an important role not only in pathogenesis but also as a prognostic factor of AMI.

Until recently, the biochemical signaling pathways involved in activation of the immune system were poorly understood. A family of Toll-like receptors (TLRs) has been defined as a key component of pathogen-associated molecular pattern recognition machinery (13). Currently 10 Toll-like receptors (TLRs) have been reported in mammalian species, and these appear to recognize distinct pathogen-associated molecular patterns controlling innate immune responses (14). When TLRs on monocyte / macrophages are activated, this leads to activation of the nuclear factor (NF)-κB pathway which brings about the production of proinflammatory cytokines and expression of co-stimulatory molecules, resulting in the induction of acquired immunity (15-17).

In this study, we summarize the current knowledge about the role of the TLR signaling pathway in the pathogenesis of ischemic coronary artery disease, and also review mechanisms of chronic inflammatory vessel disturbance as a pathogenetic pathway in atherosclerosis. The main focus is on clinical evidence, and key observations from animal experiments and basic science are included. This review begins with the local activation of the TLR signaling pathway at the coronary artery, and finally deals with the relationship between systemic inflammatory changes and the TLR signaling pathway, with acceptance of the concept that the two features are closely connected.

3. HUMAN TLR STRUCTURE AND FUNCTION

Toll was first described as a type 1 transmembrane receptor which is an integral membrane protein with a cytoplasmic domain and a large extracytoplasmic domain, and which controls the embryonic dorsal-ventral pattern of Drosophila (18). Rock’s report has identified mammalian homologues of toll receptor proteins referred to as TLR proteins (19). TLRs are constructed by two domains including an extracellular amino terminus and a carboxy terminal intracellular domain as well as Drosophila Toll protein. The extracytoplasmic domain of TLR proteins contains a varying number of leucine-rich repeat (LRR) ectodomains, which are presumably involved in ligand binding (19). The amino acid sequences of the cytoplasmic domain of TLR proteins is similar to the mammalian type 1 interleukin-1 receptor (IL1-R1) and its conserved region is called Toll / IL1-R1 domain (20). The conservation Toll / IL1-R1 signaling pathway between diverse species supports the importance of the innate immune system in host responses to pathogen attack and environmental stress (20). The Toll / IL1-R1 signaling pathway proceeds through the recruitment of adaptor protein myeloid differentiation factor (MyD)-88, which engages two putative serine / threonine kinases, IL-R1-associated kinase (IRAK)-1 and IRAK-2, to the receptor complex (21). These kinases subsequently affiliate with the adaptor protein tumor necrosis factor-associated factor (TRAF) 6, which associates them with the protein kinase nuclear factor-κB (NF-κB)-inducing kinase (NIK) (22). NIK activates the inhibitor of NF-κB (I-κB) kinase (IKK) complex, such as IKK-α, -β and -γ, which leads to the phosphorylation and degeneration of the NF-κB inhibitor protein, I-κB (23). Finally, NF-κB is released and translocates to the nucleus where it regulates the expression and secretion of many kinds of molecules including pro-inflammatory cytokines, chemokines and adhesion molecules (24).

Currently, more than 10 human TLRs have been identified, and at least 10 human homologues of Drosophila Toll have been sequenced (14). Different members of the TLR family recognize different pathogen motifs, or pathogen-associated molecular patterns (PAMPs), such as Gram-positive bacterial, mycobacterial, fungal and spirochetal cell-wall components (peptidobycan) for TLR2 (25), Gram-negative bacterial component and lipopolysaccharide (LPS) for TLR4 (26), flagellin for TLR5 (27), and CpG-DNA-repeats from the bacterial DNA for TLR9 (28).

TLR or related compounds may be important in human development and the maintenance of normal organism functioning, paralleling their proposed roles in Drosophila. It has been well demonstrated that TLR2 and TLR4 among the human TLRs are expressed in atherosclerotic vessel and ischemic heart (29, 30). In the absence of infection, these reports are consistent with the notion that a TLR2- and TLR4-mediated immune response may be involved in the pathogenesis of human ischemic heart disease.
Role of Toll like receptor signaling pathway in CAD

4. THE ROLE OF TLR IN ISCHEMIC HEART

4.1. Local expression of TLRs in atherosclerotic lesion

It has been clearly demonstrated that atherosclerotic lesion development involves the immune system during its initiation and progression (1, 2). Coronary plaque rupture coexists with numerous inflammatory mediators including proinflammatory cytokines, acute-phase reactants and soluble adhesion molecules, was associated with a low risk of cardiovascular events (31, 32). These reports suggest that activation of TLR4 signal may play an important role in the progression of atherosclerosis. It has been demonstrated that expression of TLR4 is mainly localized in infiltrating macrophages in human coronary artery specimens obtained from coronary artery specimens obtained from autopsy cases (33). In addition, TLR2 and TLR4 immunostainings are frequently colocalized with NF-κB immunostaining, which is a common downstream pathway of TLR2 and TLR4 signals, in atherosclerotic plaques obtained from patients undergoing endarterectomy (34). There is cross-talk between TLR2 and TLR4, through which TLR2 expression is regulated by TLR4 expression (35, 36). These reports suggest a close link between the progression of coronary atherosclerosis and TLR2 / TLR4 signals. An apoE-deficient mouse model has shown that loss of TLR4 and its adaptor molecule MyD88 reduces the severity of atherosclerosis and alters atherosclerotic plaque (37). Furthermore, Bjorkbacka’s report using MyD88-null mice has demonstrated that TLR4 deficiency was associated with alterations in coronary plaque composition, which reduced both lipid and macrophage contents and markedly decreased the expression of inflammatory factors including pro-inflammatory cytokines, chemokines and adhesion molecules (38). An experimental model has shown that activated macrophages within plaque are capable of degrading the extracellular matrix by secretion of matrix metalloproteinase 9, which can be stimulated by TLR4 activation and which induces plaque degradation and rupture (39, 40). It has also been reported that activated TLR4 signal induces expression of apoptotic molecules of the Fas death pathway (41). On the basis of these observations, it has been suggested that expression of TLR4 in infiltrating macrophages in the coronary arteries may be an important factor underlying coronary plaque destabilization and rupture.

On the other hand, there has been only limited information on the role of TLR2 in the process of atherosclerosis. Mullick et al. have demonstrated the importance of TLR2 expression in non-bone marrow-origin cells such as endothelial cells in lesion formation by use of bone marrow transplantation in low-density lipoprotein receptor knock-out mice deficient in TLR2 (42). Therefore, this report suggests a role for TLR2 signal in modulating the severity of experimental atherosclerosis (42). It has also been demonstrated that an exogenous TLR2 specific ligand is present in atherosclerotic plaques from human coronary arteries and TLR2 stimulation increased inflammatory markers, such as proinflammatory cytokines and chemokines, in human adventitial fibroblasts in vitro (43). From these observations, local arterial TLR2 stimulation induced neo-intima and atherosclerotic plaque formation in an animal artery, and may be an important mediator in arterial occlusive disease such as coronary heart disease. Indeed, the role of TLR2 and TLR4 in the innate immune response to PAMPs is clearly established and a signaling pathway involving these receptors also provides a plausible link between the innate and adaptive immune systems, because both TLR2 and TLR4 use common downstream signaling pathways via MyD88 and NF-κB pathway (44). An animal model with transfected TLR2 and TLR4 has shown that local overexpression of TLR2 and TLR4 at the vessel wall synergistically accelerates atherosclerosis and increases the expression of adhesion molecules (vascular cell adhesion molecule 1 and intercellular adhesion molecule 1) and chemokine (MCP-1) (29). This report has also demonstrated that transfection of TLR2 and TLR4 resulted in synergistic activation of NF-κB signal, which is a common downstream pathway, in the vessel wall in vivo and in vascular smooth muscle cells in vitro (29).

It has been hypothesized that activation of TLR4 signal in coronary artery may act to promote TLR2 signal via a synergistic effect, and may lead to production of several inflammatory mediators, such as proinflammatory cytokines, chemokines and adhesion molecules. These accelerate the progression of coronary plaque instability and rupture, via MyD88 and NF-κB signal, leading finally to coronary artery occlusion. These results suggest that the functional link between activated TLR4 and TLR2 signals plays a pivotal role in the pathogenesis of ischemic heart disease.

4.2. Systemic expression of TLRs in ischemic heart disease

Our previous study has shown an increase in TLR4 (both mRNA and protein) levels in circulating monocytes obtained from patients with AMI (10). In addition, TLR4 generation capacity in circulating monocytes is higher in AMI patients with heart failure than in those without heart failure, and is related to secretion of proinflammatory cytokines (10). These results suggest that activated TLR4 signal is involved in the monocyte inflammatory response to myocardial ischemic injury and may potentially drive heart failure after AMI. In agreement with our previous study, Methe’s report has demonstrated that activation of monocyte TLR4 signal is related to the downstream release of inflammatory cytokines in patients with acute coronary syndrome (45). An increase in TLR2 levels as well as TLR4 levels has been shown in PBMC from patients with acute coronary syndrome (46). Although TLR2 and TLR4 have been identified as receptors for exogenous ligands including bacterial component and LPS, recent studies have demonstrated that...
Role of Toll like receptor signaling pathway in CAD

Figure 1. The hypothetical role of TLR2 and TLR4 signals in ischemic heart disease. Coronary risk factors (hypertension, diabetes, hyperlipidemia, obesity) increase exposure to fatty acids, oxidative stress and HSP as endogenous TLR ligands. In addition, Chlamydia HSP60 and LPS activate TLR2 and 4 signals as exogenous TLR ligands. Local expression of TLR2 and TLR4 in the coronary artery induce the release of any inflammatory mediators (cytokine, chemokine and adhesion molecules) and coronary plaque instability. Systemic expression of TLRs is induced by any endogenous ligands in response to ischemic heart, and induce left ventricular remodeling and dysfunction and arterial remodeling.

the TLR signaling pathway is activated by endogenous ligands, such as oxidative stress, oxidized low-density lipoprotein (ox-LDL) and heat shock protein (HSP) (47-49). In particular, HSP may be released after myocardial ischemic change as noninfectious endogenous danger signals, thus activating a systemic inflammatory reaction. Among HSPs, HSP70 is a potent endogenous activator of the innate immune system as a common endogenous ligand of both TLR2 and TLR4, and is capable of stimulating inflammatory cytokine, chemokine and adhesion molecule production by the monocyte-macrophage system (50, 51). Asea et al. have reported that the release of HSP70 in response to ischemic myocardium may activate TLR2 and TLR4 signals in circulating monocytes (50). Dybdahl et al. have recently shown that circulating HSP70 is related to the extent of myocardial damage, and may play a role in the systemic inflammatory response against ischemic myocardial injury (52). Our previous study has demonstrated that circulating monocytes release HSP70, which is a potent endogenous ligand of monocyte TLR4 signal, in response to myocardial ischemic damage (53). On the other hand, serologic evidence has reported a link between an unusual species of Chlamydia pneumoniae and the pathogenesis of ischemic heart disease in patients with AMI and coronary heart disease (54). Recent studies suggest that Chlamydia HSP70 and its LPS induce oxidative modification of LDL, and are recognized by TLR2 and TLR4 signal as exogenous TLR ligand (55, 56). TLR4 signaling upregulates TLR2 in LPS-stimulated macrophages, suggesting that the cross-talk between TLR2 and TLR4 works as a positive feedback loop (57). By activating a positive feedback signal, TLR4-TLR2 cross-talk may lead to an amplification of monocyte activation and immune response via inflammatory mediator production. It is therefore speculated that monocyte TLR4 signal in response to ischemic myocardial injury may mediate TLR2 signal as a positive feedback mechanism and may be involved in activation of the immune response in patients with ischemic heart disease.

4.3. The close link between local and systemic TLR signals in ischemic heart

The hypothetical role of TLR2 and TLR4 signals in ischemic heart disease is shown in the Figure (Figure 1). Several reports have demonstrated that
Role of Toll like receptor signaling pathway in CAD

activation of peripheral monocytes is involved in adverse outcomes and adverse left ventricular remodelling after AMI (7, 8). An epidemiological study has demonstrated that increases in several inflammatory markers including TNF-α, IL-6 and C-reactive protein are independent predictors of devastating cardiovascular events in the older population without cardiovascular disease (58). Our previous study demonstrated that an increase in TLR4 levels and downstream release of inflammatory cytokines, such as IL-6, GM-CSF and TNF-α, in circulating monocytes may be involved in the development of heart failure after AMI (10). In addition, an increase in circulating levels of HSP70 is associated with the TLR4 signal-mediated immune response, left ventricular dysfunction and the presence of heart failure after AMI (53). A recent report suggests that soluble TLR2 levels may be involved in the innate immune response in the pathogenesis of heart failure after AMI (59). These reports suggest that monocyte-related inflammatory mediators are released by the activation of systemic TLR2 and TLR4 signals into the systemic circulation, and are associated with certain molecular, clinical and physiological aspects of heart failure, including but not limited to progression of left ventricular dysfunction, left ventricular remodeling and myocyte apoptosis in transgenic mice with overexpressing inflammatory mediators (60, 61). In addition, cardiac events including recurrent ischemic heart disease and cardiac death are mostly precipitated by rupture or erosion of structurally weakened coronary plaque (62). Arterial remodeling has recently been thought to be the main cause of the prevalence of vascular pathologies due to rupture or erosion of coronary plaque (63). It has also been reported that the immune response via TLR signal is involved in arterial remodeling in a animal model (64). We can therefore speculate that systemic TLR2 and TLR4 signals may be related to activation of local TLR2 and TLR4 signals via a positive feedback loop and may be involved in the pathogenesis of adverse arterial remodeling which may cause AMI and cardiac death.

A mouse model of MI has recently shown that inhibition of TLR4 by its antagonist attenuates the inflammatory response to MI as evidenced by a significant reduction in infarct size and decreased expression of inflammatory mediators (65). It has been reported that a new benzisothiazole derivative, which inhibits TLR4 signal transduction, suppresses LPS-induced upregulation of cytokines, adhesion molecules and procoagulant activity in human vascular endothelial cells and peripheral mononuclear cells. This suggests that this compound may inhibit the progression of atherosclerosis (66). TLR4 signal may therefore represent a significant target molecule for the design of specific inhibitors as a novel therapeutic agent to combat the progression of ischemic heart disease.

5. SUMMARY

On the basis of current knowledge, the role of at least TLR2 and TLR4 signals in the immune response to any coronary risk factors appears to be established. The TLR signaling pathway may also provide a plausible link between the immune response and the pathogenesis of human ischemic heart disease. Further studies are needed to determine whether inhibition of TLR signal-mediated immunity will reduce the progression of atherosclerosis and the risk of ischemic heart disease in humans.

6. ACKNOWLEDGEMENTS

This study was supported by a grant from the Keiryokai Research Foundation (No. 98) and the Open Translational Research Center, Advanced Medical Science Center, Iwate Medical University.

7. REFERENCES

Role of Toll like receptor signaling pathway in CAD

marker of the extent of cardiac injury. Cardiology, 90, 52-57 (1998)


Role of Toll like receptor signaling pathway in CAD


Role of Toll-like receptor signaling pathway in CAD


**Key Words:** Arterial remodeling; heat shock protein 70; inflammatory cytokine; ventricular remodeling; vulnerable coronary plaque

**Send correspondence to:** Mamoru Satoh, Department of Internal Medicine II and Memorial Heart Center, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka 020-8505, Iwate, Japan, Tel: 81-19-651-0401, Fax: 81-19-651-0401, E-mail: m_satoh@imu.ncvc.go.jp

http://www.bioscience.org/current/vol13.htm