J. Toxicol. Pub. Health
Vol. 23, No. 2, pp. 97-105 (2007)

Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

Perspectives - Minireview

Toll-like Receptors in Host Defense and Immune Disorders

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Received May 30, 2007; Accepted June 19, 2007

Toll-like receptors (TLRs) play a crucial role in initiating and regulating innate and adaptive immune responses by detecting invading microbial pathogens. TLRs can also respond to non-microbial molecules derived from damaged tissue. Accumulating evidence suggests that deregulation of TLRs results in the dysfunction of immune system and ultimately increases the risk of many immune and inflammatory diseases including infectious diseases, allergy, and autoimmune diseases. Therefore, understanding how the immune system is controlled by TLRs will provide new insight to find the way to prevent or treat infectious diseases and immune disorders.

Key words: Toll-like receptor, Immune dysfunction, Inflammation, Infection, Innate and Adaptive immunity.

INTRODUCTION

An immune system has been evolved to protect an organism against infection by detecting and eliminating invading pathogens. In general, the immune systems of vertebrates such as human consist of innate immunity and adaptive immunity. The innate immunity provides an instant response to infection with broad reactivity as the first line of host defense system. The adaptive immunity responds with a high degree of specificity for the recognition of the pathogen. While the adaptive immune response to the initial exposure to an antigen requires several days to be mounted, immunological memory enables adaptive immune cells to be activated quicker and stronger. Alterations in immune function can cause adverse or immunotoxic effects such as immunosuppression and overactivation eventually contributing to the development of chronic diseases. When the immune system is suppressed, impairment of host immunity to invading pathogens results in the increased susceptibility to life-threatening infections and neoplastic diseases. In contrast, hyperactive immune responses can culminate in autoimmune diseases, hypersensitivity, and chronic inflammation leading to unnecessary tissue damage.

Toll-like receptor (TLR) is one of the pattern-recognition receptors (PRRs) detecting pathogen-associated molecular patterns (PAMPs) derived from invading microorganisms. The activation of TLRs and downstream signaling pathways culminates in the expression of immune and inflammatory mediators. As a result, TLRs play an important role in host defense system by initiating and regulating innate and adaptive immune responses (Medzhitov and Janeway, 2000). This paper will review the role of TLRs in host defense and how deregulation of TLRs can affect immune dysfunction and risk of the relevant diseases.

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TOLL-LIKE RECEPTORS REGULATING INNATE AND ADAPTIVE IMMUNE RESPONSES

Toll, a protein identified in *Drosophila*, is known to be involved in the dorsal-ventral patterning in the developmental stage of embryos. In adult Drosophila, Toll participates in the regulation of immune responses by the production of anti-fungal peptide, drosomycin (Lemaitre *et al.*, 1996). TLR, the mammalian homolog of Drosophila Toll protein, is a type 1 transmembrane receptor consisting of an extracellular domain with leucine-rich repeat (LRR) motifs, a transmembrane domain, and a cytoplasmic Toll/IL-1R (TIR) homology domain (Medzhitov *et al.*, 1997). A constitutively active TLR4 induced NFκB activation and the expression of inflammatory cytokines and co-stimulatory molecules in human cell lines suggesting the role of TLRs in inducing immune responses in mammalian system (Medzhitov *et al.*, 1997). TLRs are primarily expressed in antigen-presen-

ing cells (APCs) such as macrophages and dendritic cells (DCs). Recognition of invading pathogens by TLRs elicits a maturation process in DCs to induce the expression of co-stimulatory molecules such as CD80 and CD86 and the production of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-12 (Medzhitov, 2001). Matured DCs present microorganism-derived peptide-MHC complex to naïve T cells to initiate antigen-specific adaptive immune responses. Activation of naïve $CD4^+$ T cells by DCs leads to the differentiation of T cells into effecter cells, either Th1 or Th2 cells. The fate of T cells depends on various factors in the microenvironment such as the type of DC population, the isotype of TLR expressed and activated on DCs, and the type of cytokines produced by DCs (Iwasaki and Medzhitov, 2004). Distinct subsets of DCs elicit different responses to TLR agonists, which may modulate T cell differentiation. Plasmacytoid DCs stimulated with TLR7 or TLR9 agonists induce the production of type 1 interferons (IFNs) whereas myeloid DCs produce IL-12 in response to TLR7 or TLR9 agonists (Schnare *et al.*, 2001; Hemmi *et al.*, 2003). Different TLRs differentially regulate the Th1/Th2 response. In general, activation of TLR4 and TLR9 in DCs results in a skewed Th1 response (Pulendran *et al.*, 2001; Krieg, 2000). While activation of wild-type DCs by TLR4 agonist induces production of IFN-γ, Th1 cytokine in lymphocytes, deficiency DCs deficient of MyD88, an adaptor signaling molecule of TLRs, enhances the showed the enhanced production of Th2 cytokine, IL-4, demonstrating that TLR4 signaling can activate DCs to support Th2 immune responses through MyD88-independent pathway (Kaisho *et al.*, 2002). The live attenuated yellow fever vaccine, YF-17D, which activates various TLRs, TLR2, 7, 8, and 9 in DCs elicits a mixed Th1 and Th2 response (Querec *et al.*, 2006). MyD88-deficient mice injected with YF-17D show a reduction in production of IFNγ whereas YF-17D treatment to TLR2-deficient mice enhanced IFNγ production (Querec *et al.*, 2006) suggesting that TLRs are involved in regulating of Th1/Th2 balance. Therefore, it has now become apparent that TLRs play a critical role in the instruction and control of innate and adaptive immune responses (Iwasaki and Medzhitov, 2004).

MICROBIAL AGONISTS AND SIGNALING PATHWAYS OF TOLL-LIKE RECEPTORS

TLRs are germline-encoded receptors with limited number of isotypes. Currently, at least thirteen of mammalian TLRs have been identified. Each isotype of TLR recognizes different microbial components with specificity (Table 1). The genetic study using mice hyporesponsive to lipopolysaccharide (LPS), a gram-negative bacterial component, revealed that a mutation in TLR4, either spontaneous knockout (C57BL/10ScCr) or replace-

Table 1. Toll-like receptors and their agonists

Receptor	Microbial agonists	Host-derived agonists
TLR1 (with TLR2)	Triacyl lipopeptides	
TLR2	Peptidoglycan	Heat-shock protein 70
	Lipoteichoic acid	Saturated fatty acids
	GPI-linked protein	
	Zymosan	
	Lipoproteins	
	Atypical lipopolysaccharide	
TLR3	Double-stranded RNA	Double-stranded RNA
TLR4	Lipopolysaccharide	Heat-shock protein 60/70
	Fusion protein	Fibronectin
	(Respiratory syncytial virus)	Hyaluron
		Heparan sulfate
		Fibrinogen
		Saturated fatty acids
		Oxidized LDL
TLR5	Bacterial flagellin	
TLR6 (with TLR2)	Diacyl lipopeptides	
TLR7 and TLR8	GU-rich single-stranded RNA	Single-stranded RNA
TLR9	DNA (Bacterial or viral)	DNA
TLR10 (human only)	Unknown	
TLR11 (mouse only)	Uropathogenic bacteria	
	Protozoan profillin-like protein	
TLR12 (mouse only)	Unknown	
TLR13 (mouse only)	Unknown	

ment of proline with histidine at position 713 (C3H/HeJ), confers the resistance to LPS exposure (Poltorak *et al.*, 1998). This study showed that TLR4 is the receptor for bacterial LPS and first demonstrated that TLRs are able to recognize microorganism-derived substances. TLR4 also recognizes the viral invasion such as respiratory syncytial virus (Kurt-Jones *et al.*, 2000). Bacterial lipopeptides and peptidoglycan are detected by TLR2. TLR5 senses flagellin, a component of bacterial flagella filament from Gram(+) and Gram(-) bacteria (Hayashi *et al.*, 2001). TLR3 responds to double-stranded RNA which is generated during viral replicative cycle. Polyinosine-polycytidylic acid (poly(I:C)) is an analog of dsRNA used as the synthetic ligand for TLR3 (Alexopoulou *et al.*, 2001). TLR7 and TLR8 recognize GU-rich short single-stranded RNA derived from virus (Heil *et al.*, 2004) and small synthetic molecules such as imidazoquinolines and nucleoside analogues (Hemmi *et al.*, 2002). TLR9 detects unmethylated CpG oligonucleotides derived from bacteria and virus.

TLR4 stimulated by LPS or lipid A undergoes homodimerization to initiate and amplify the activation of intracellular signaling pathway (Zhang *et al.*, 2002; Saitoh *et al.*, 2004). TLR2 is required to form a heterodimer with TLR1 or TLR6 depending on the nature of TLR2 agonist. Diacylated lipopeptides activate TLR2 and TLR6 dimers whereas triacylated lipopeptides are detected by TLR2 and TLR1 heterodimers (Takeuchi *et al.*, 2001, 2002). Acylation with saturated fatty acids such as lauric acid, myristic acid and palmitic acid is critical for agonistic activity of certain TLR2 and TLR4 ligands (Munford and Hall, 1986). Deacylation of saturated fatty acid moiety or replacement with unsaturated fatty acids is one of the mechanisms to inactivate the ligand activity (Kitchens *et al.*, 1992; Krauss *et al.*, 1989; Lu *et al.*, 2005). Furthermore, treatment with saturated fatty acids to immune cells led to the activation of TLR2 and TLR4 and the expression of immune-mediators (Lee *et al.*, 2001, 2004).

In addition to the receptor dimerization, certain TLRs require co-receptors for microbial recognition and activation of signaling pathways. TLR4 forms a complex with glycoproteins such as CD14 and MD2 upon agonist stimulation. CD14 is present as both a soluble form and a membrane-bound form and binds to LPS. Smooth LPS, but not rough LPS, cannot bind to TLR4 without CD14. For rough LPS, CD14 is required for the activation of TRIF/TRAM-dependent signaling pathway (Jiang *et al.*, 2005). MD2 is a 25- to 30-KDa soluble protein and associated with TLR4. MD2 knockout mice do not respond to LPS showing that MD2 is indispensable to recognize LPS (Nagai *et al.*, 2002). CD36 is an 88-KDa

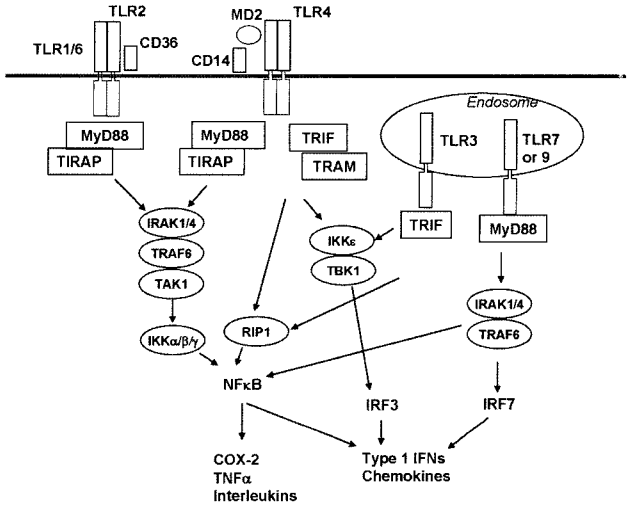

Fig. 1. Downstream signaling pathways of Toll-like receptors. Toll-like receptors (TLRs) recruit different combination of adaptor molecules, MyD88, TIRAP, TRIF, and TRAM. Two major signaling pathways of TLRs are MyD88-dependent and MyD88-independent pathways (TRIF-dependent) leading to the activation of downstream kinases. The consequent activation of transcription factors including NFκB, IRF3, and IRF7 culminates in the expression of immune and inflammatory mediators.

glycoprotein and participates in the recognition of macrophage-activating lipopeptide-2 (MALP-2) and lipoteichoic acid to activate TLR2/TLR6 dimer (Hoebe *et al.*, 2005). UNC-93B is a 12-membrane-spanning protein and found in the endoplasmic reticulum (Tabeta *et al.*, 2006). Macrophages derived from mice with a missense mutation in UNC-93B showed defective responses to the TLR3, 7, and 9 agonist.

The activation of TLRs recruits adaptor molecules to the cytosolic TIR domain such as myeloid differentiation factor 88 (MyD88), Toll-interleukin 1 receptor (TIR) domain-containing adapter protein (TIRAP/Mal), Toll/IL-1 receptor (TIR)-domain-containing adaptor inducing IFNβ (TRIF), and TRIF-related adaptor molecule (TRAM). Different TLRs have different combination of adaptors leading to the activation of two major downstream signaling pathways, MyD88-dependent and -independent (TRIF-dependent). The signaling pathways of TLRs are illustrated in Fig. 1. MyD88 is the common adaptor used by most TLRs except TLR3 (Medzhitov *et al.*, 1998). TIRAP is recruited to TLR2 and TLR4 to cooperate with MyD88. TLR3 and TLR4 activation requires TRIF which is responsible for MyD88-independent NFκB and mitogen-activated protein kinases (MAPKs) activation. MyD88-dependent signaling pathways are activated NFκB activation occurs in an earlier and faster period whereas TRIF-dependent signaling pathways

NFκB activation showed a delayed kinetics in TLR4 signaling. TRAM named as TIR domain-containing protein (TIRP) and TIR-containing adaptor molecule-2 (TICAM-2), is associated with TLR4 and TRIF, but not other TLRs (Fitzgerald *et al.*, 2003). TLR5, TLR7, and TLR9 have MyD88 as an adaptor.

MyD88 associates with cytosolic domain of TLRs through TIR-TIR homophilic interaction and recruits IL-1 receptor-associated kinases (IRAK-4 and IRAK-1) through a death domain (DD) interaction. This initiates phosphorylation and subsequent degradation of IRAK-1 and association with tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) leading to the activation of IKKβ and MAPKs. As a result, transcription factors, NFκB and AP-1, are activated to induce the expression of inflammatory and immune-related gene products.

TRIF interacts with RIP1 which is the kinase known to activate NFκB. In addition, TRIF activates TBK1 and IKKε which phosphorylate and activate IRF3 and IRF7 leading to the expression of type 1 interferons (IFNs) including IFNβ in TLR3 and 4 signaling pathways (Kawai *et al.*, 2001; Toshchakov *et al.*, 2002). IFNβ activates an IFNα/β receptor and downstream signaling pathways, including JAK kinases in an autocrine/paracrine manner leading to the phosphorylation of STAT1 and the expression of IFN-inducible genes such as iNOS and IP-10 (Brierley and Fish, 2002; Gao *et al.*, 1998). Src-family tyrosine kinases are also involved in the regulation of the expression of IFN-inducible genes in TRIF-signaling pathways of TLR4 (Lee *et al.*, 2005).

TLR7 and TLR9 can produce type 1 IFNs without TRIF through the interaction between MyD88, IRAK-1, and IRF7 in plasmacytoid dendritic cells (Honda *et al.*, 2005). The production of type 1 IFNs by the activation of TLRs including TLR3, 4, 7 and 9 recognizing viral components is critical for anti-viral defense mechanism.

MODULATION OF SUSCEPTIBILITY TO MICROBIAL INFECTION BY TOLL-LIKE RECEPTORS

Since TLRs play an important role in mounting and regulating innate and adaptive immune responses against microbial infection, the activation of TLRs could be beneficial to enhance host resistance to bacterial and viral infection. Indeed, the treatment of a new class of synthetic lipid A mimetics, the aminoalkyl glucosaminide 4-phosphates (AGPs), of which activity is dependent on TLR4, elicits protective immune responses to bacteria (*Listeria monocytogenes*) or influenza virus infection (Cluff *et al.*, 2005). On the other hand, the defect in the activation process of certain TLRs could have deleterious impacts on host resistance resulting from dysfunction of immune cells against pathogen invasion. Considerable data show that the impairment of TLR activation increases the risk of infectious diseases.

Mice having a cytoplasmic mutation in TLR4 (C3H/HeJ) were more susceptible to infection with gram-negative bacteria, *Salmonella typhimurium*, *Escherichia coli*, and *Neisseria meningitides* than wild-type mice (O'Brien *et al.*, 1980; Hagberg *er al.*, 1984; Woods *et al.*, 1988). TLR2-deficient mice infected with *Streptococcus pneumoniae*, gram-positive bacteria, had higher bacterial burden in brain and earlier kinetics of death than wild-type mice resulting from reduced brain bacterial clearance (Echchannaoui *et al.*, 2002). Mice with TLR9 missense mutation exhibited increased susceptibility to cytomegalovirus infection (Tabeta *et al.*, 2004). The deficiency of TLR also contributes to the impairment of bactericidal function of macrophages. TLR2-deficient polymorphonuclear leukocytes showed delayed phagocytosis process and lower oxidative bactericidal activity for *Streptococcus pneumoniae* infection (Letiembre *et al.*, 2005).

Individuals with TLR4 mutation (Asp299Gly/Thr399Ile) which makes a less effective TLR4 had higher risk of gram-negative infection and were more prone to develop septic shock (Lorenz *et al.*, 2002; Agnese *et al.*, 2002). Rare heterozygous missense mutations of TLR4 were associated with enhanced risk of meningococcal sepsis (Smirnova *et al.*, 2003). Arg753Gln mutation of TLR2 which reduces the responsiveness of lipopeptide agonists from *Borrelia burgdorferi* and *Treponema pallidum* was associated with staphylococcal infections (Lorenz *et al.*, 2000). The frequency of TLR2 mutation of Arg753Gln was higher in tuberculosis patients than in healthy controls (Ogus *et al.*, 2004). TLR2 with Arg677Trp mutation did not activate NFκB in response to *Mycobacterium leprae* and *Mycobacterium tuberculosis* (Bochud *et al.*, 2003) and this polymorphism was more found in the patients with lepromatous leprosy and tuberculosis (Ben-Ali *et al.*, 2004; Kang and Chae, 2001). These suggest that dysfunction of TLR2 increases the susceptibility of developing certain infectious diseases such as lepromatous leprosy and tuberculosis.

Heat shock protein gp96, a chaperone for TLRs, is involved in the activation of various TLRs including TLR2, 4, 5, 7, and 9. Gp96-deficiency in macrophages greatly increased the susceptibility of mice to *Listeria monocytogenes* infection (Yang *et al.*, 2007).

Certain viruses devise mechanisms to escape host defense system by inhibiting TLR signaling. Vaccina

virus produces A46R, A52R, and N1L proteins to disrupt TLR activation through the interaction of TLR signaling components (DiPerna *et al.*, 2004; Stack *et al.*, 2005; Harte *et al.*, 2003). A46R which contains TIR domain associates with TLR adaptors through TIR-TIR interaction. A52R targets with IRAK2 and TRAF6 while N1L interacts with TBK1.

IMMUNE DISORDERS ASSOCIATED WITH OVERACTIVATION OF TOLL-LIKE RECEPTORS

Immune system should be orchestrated under tight control to maintain the balance between down-regulation and over-stimulation of immune responses. The overactivation of TLRs by microbial or host-derived molecules may result in the hyper-responsiveness of immune system and contribute to the development of chronic inflammatory diseases and various immune disorders.

Sepsis. Since TLR activation elicits the production of inflammatory mediators, hyperactivation of TLR signaling may result in excessive and prolonged inflammatory responses. Inappropriate and uncontrolled inflammation is closely linked to the increased risk of the development of inflammatory diseases. Sepsis induced by bacterial infection is a clinical syndrome accompanied by systemic inflammatory responses due to excessive production of inflammatory mediators. Because of frequent incidences and high mortality, it has been considered as one of the most critical complications derived from microbial infection. Microbial components such as LPS, lipopetides, unmethylated DNA, and flagellin evoke septic symptoms mediated through the activation of TLR signaling and consequent expression of inflammatory gene products including TNFα, IL-1β, iNOS, and COX-2 in macrophages and monocytes (Beutler, 2004).

Allergy and asthma. There has been a considerable increase in the prevalence of allergies including asthma in the past decades. This is at least partly due to the increasing exposure of various chemicals and environmental pollutants as the countries have been developed. Allergies can be also caused by increasing use of antibiotics and vaccination which affect immune system. Majority of asthma incidence in adults and children is triggered by respiratory infections of viruses. Excessive immune and inflammatory responses in bronchial airways are important pathological symptoms of asthma patients. TLRs recognize pathogen invasion in airways and pulmonary tissues and evoke the production of immune and inflammatory mediators. Several evidences suggest the relevance of TLRs with allergy and asthma. The exposure to LPS exacerbates the symptoms of asthma. The severity of asthma in patients allergic to house dust mite was correlated better with levels of endotoxin than with mite allergen (Michel *et al.*, 1996). Pre-exposure to allergen enhanced the inflammatory responses induced by LPS (Eldridge and Peden, 2000). The national survey in United States housings showed significant correlations between endotoxin levels in houses and increased diagnoses of asthma (Thorne *et al.*, 2005). People with TLR4 G299/I399 polymorphism showed a lower risk of asthma whereas the symptom of asthma was significantly increased with elevated endotoxin levels in house dust (Werner *et al.*, 2003). There is a contrasting report that early childhood exposure to household LPS protects against development of allergies later in life (Braun-Fahrlander *et al.*, 2002). Therefore, the timing of LPS exposure seems to be important to determine if LPS can exacerbate or protect asthma symptoms. Farmers' children with a TLR2 polymorphism (a T allele in TLR2/-16934) had less incidence of a diagnosis of asthma and allergy suggesting that the genetic variation in TLR2 is associated with the susceptibility to asthma and allergies in children of farmers in Europe (Eder *et al.*, 2004). TLR9 polymorphism with a C allele at -1237 was related to the increased risk for asthma in European Americans, but not African Americans (Lazarus et al., 2003). These studies suggested that TLR activation can be one of the critical factors to modulate the pathogenesis of asthma and allergies.

Autoimmune diseases. Overactive immune responses can result in the dysfunction of immune system inducing autoimmune disorders in which immune system produces autoantibodies against self antigens. TLRs can respond to endogenous host-derived components as well as exogenous microbial pathogens (Marshak-Rothstein, 2006) as depicted in Table 1. TLR2 and TLR4 can be activated by damage-associated molecular pattern molecules (DAMPs) derived from damaged cells and injured tissue including heparan sulfate, fibronectins, heat shock proteins, saturated fatty acids, and modified low-density lipoprotein (Lee and Hwang, 2006; Seong and Matzinger, 2004). TLR3 and TLR7 can be stimulated by host-derived single-stranded RNA (Lau *et al.*, 2005; Kariko *et al.*, 2004) and TLR9 can recognize mammalian DNA (Leadbetter *et al.*, 2002). Cell necrosis, tissue injury, and inflammation can promote the release of these endogenous TLR agonists which stimulate immune responses as self-antigens.

Patients of systemic lupus erythematosus (SLE), one of the most common autoimmune diseases, produce autoantibodies against nuclear self-antigens including DNA, histones, and chromatin. Immune complexes containing self DNA activate B cells expressing an antigen receptor specific for self-immunoglobulin-gamma (IgG) mediated through the synergistic activation of the antigen receptor and TLR9 (Leadbetter *et al.*, 2002). The high-mobility group box 1 (HMGB1), a nuclear DNA-binding protein, released from damaged cells is associated with DNA-containing immune complex and augments cytokine production through TLR9 activation (Tian *et al.*, 2007). These suggest that TLR signaling may contribute the development and pathogenesis of autoimmune diseases by recognizing endogenous self-antigens.

CONCLUDING REMARK

TLR signaling is required to defend host against microbial infection. The impairment of TLR activation results in the immunodeficiency or immunosuppression which renders host vulnerable to infectious diseases. On the other hand, excessive activation of TLR signaling may lead to autoimmunity, allergy, or chronic inflammation resulting in the damage to the host. Therefore, it will be critical to identify TLR modulators derived from microbial components, host-derived molecules, chemicals and drugs, and to understand the underlying mechanisms. This will help us to find the beneficial way to regulate TLR activity in order to harmonize innate and adaptive immune systems.

ACKNOWLEDGEMENT

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-331-E00425) and a grant (07142KFDA583) from Korea Food & Drug Administration in 2007.

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