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Toll-like Receptors

Ho Sik Shin[1*], Yeon Soon Jung[1] and Hark Rim[1]

[1]Department of Internal Medicine, Kosin University College of Medicine, Busan, Korea.

Authors' contributions

This work was carried out in collaboration between all authors. Author HSS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors YSJ and HR managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Mammals sense pathogen invasion through pattern-recognition receptors (PRRs). A group of transmembrane proteins, Toll-like receptors (TLRs) are mainly expressed on antigen-presenting cells, such as macrophages or dendritic cells, and play critical roles as PRRs (1). TLR signaling activates antigen-presenting cells that provoke innate immunity and establish adaptive immunity. TLRs can be activated not only by invading pathogens but also by certain danger or stress-associated endogenous molecules leading to the induction of sterile inflammation. Activation of TLRs is a first line defense of the immune system, leading not only to the activation and recruitment of neutrophils and macrophages to sites of infection, but also to the enhancement of antimicrobial activity (2). Each TLR has common effects, such as inflammatory cytokine induction or upregulation of costimulatory molecule expression. However, TLRs also have specific functions, exemplified by type I IFN-inducing ability. These immunoadjuvant effects are critical in antimicrobial immunity and also involved in manifestations of autoimmunity (1). Therefore, understanding the molecular mechanisms of TLRs should facilitate the development of therapeutic solutions for allergy and autoimmune diseases.

Keywords: Toll-like receptors; innate immunity; adaptive immunity.

**Corresponding author: Email: 67920@naver.com;*

1. INTRODUCTION

Host defense in mammals copes with pathogens through innate and adaptive immunity. Both components recognize invading microorganisms as non-self and trigger immune responses to eliminate the invaders (Table 1). The innate and adaptive components have been characterized independently, and the main research interest in the immunology field has been confined to acquired immunity. B and T lymphocytes utilize antigen receptors such as immunoglobulins and T cell receptors to recognize non-self. The mechanisms by which these antigen receptors recognize foreign antigens have been intensively analyzed and major mechanisms, such as diversity, clonality and memory, have been well characterized [3]. Innate immunity functions as a pathogen sensor and contributes to the eradication of pathogens and the establishment of adaptive immunity. These functions depend heavily on pattern recognition receptors (PRRs), a group of transmembrane proteins. Toll-like receptors (TLRs, are characterized by their potent immunoadjuvant abilities to activate antigen-presenting cells (APCs) [1,4]. TLR signaling activates APCs to support TH1 cell differentiation (Fig. 1). Blocking or augmenting TLR function can modify TH1/TH2 balance and manipulate a variety of immune disorders, such as cancer, allergies, and autoimmunity [1]. Since the discovery of TLRs, rapid progress has been made in understanding the molecular and biochemical mechanisms of innate immunity, which defends the body from microbial infection by initiating inflammation and orchestrating the acquired immune response [4]. Although TLRs are crucial for innate immunity, they are also required for the induction of adaptive immune responses for combating many types of infections [2]. In this paper, I will summarize not only TLR signal transduction, but also the current knowledge regarding TLR function.

Table 1. Differences between activation of innate and adaptive immune responses [13]

	Innate Immunity	Adaptive Immunity
Recognition receptors		
type of receptors	Pattern recognition receptors (complement, mannose, immunoglobulins, TLR)	T cell receptors, B cell receptors
clonality of receptors	Non-clonal	Clonal
genetical structure	Single gene	Encoded in gene segments
receptor rearrangement	Not required	Required
recognition patterns	Conserved molecular patterns	Details of (secondary) structure
Self-foreign discrimination	Selected by evolution	Selected individually
Time to effector activation	Immediate activation	Delayed activation
Effector response	Opsonization, activation of complement and coagulation cascades, phagocytosis, proinflammatory cytokines and chemokines	Clonal expansion or anergy of antigen-specific B and T cells

2. TLR STRUCTURE AND LOCATION

2.1 TLRs are *Bona Fide* Pattern Recognition Receptors

PRRs are functionally classified into two classes: nonsignaling and signaling PRRs. Nonsignaling PRRs include soluble factors or transmembrane proteins. Acute-phase proteins, such as C-reactive protein or lectins, are important soluble molecules that can bind

to invading microorganisms. Transmembrane proteins, such as scavenger receptors, also bind to microorganisms. Signaling PRRs include transmembrane and cytosolic proteins. TLRs are representative of transmembrane-signaling PRRs. The nucleotide binding oligomerization domain (NOD) molecules Nod1 and Nod2 are cytosolic signaling PRRs [1].

Toll was first described for its involvement in innate immunity in *Drosophila melanogaster*. Fruitflies with mutant Toll receptors demonstrated high susceptibility to fungal infection [5]. One year later, a mammalian homolog of the Toll receptor (now called TLR4) was shown to induce the expressions of genes involved in inflammatory response [6]. After the characterization of the first mammalian TLR, TLR4 and several proteins that are structurally related to TLR4 were identified and named Toll-like receptors [7].

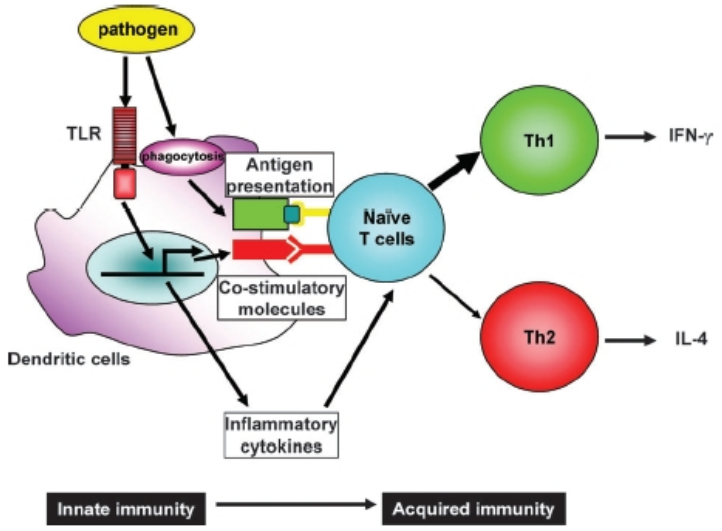

Fig. 1. Innate and adaptive immunity. Innate immune cells, such as dendritic cells and macrophages, engulf pathogens by phagocytosis, and present pathogen-derived peptide antigens to naı̈ve T cells. In addition, TLRs recognize pathogen-derived components and induce expression of genes, such as co stimulatory molecules and inflammatory cytokines. Phagocytosis-mediated antigen presentation, together with TLR-mediated expression of co-stimulatory molecules and inflammatory cytokines, instruct development of antigen-specific adaptive immunity, especially Th1 cells [3].

2.2 Structural similarity to the interleukin-1 receptor (IL-1R)

TLRs belong to a superfamily called the Toll/IL-1 receptor (TIR) family, in which all members contain cytoplasmic TIR domains. Fig. 2 shows the high level structural similarities between IL-1R and TLR.

2.3 Types of TLRs and their Locations in the Cell

The specific locations of TLRs remain unclear. For example, the first group (TLRs 1, 2, 4, 5 and 6) is found in the plasma membrane. The second group (TLRs 3, 7, 8 and 9), which is intracellular, probably signals from acidic endosomes (Fig. 3).

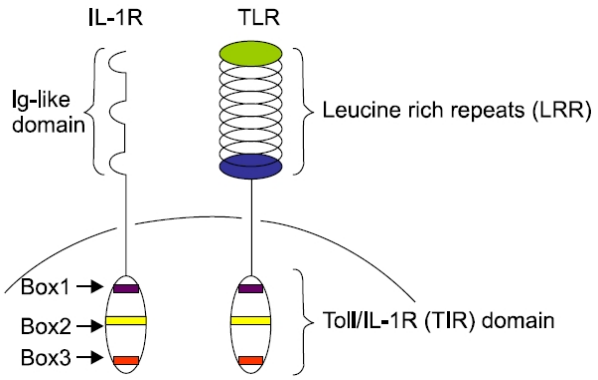

Fig. 2. High level structural similarity between IL-1R and TLR. Both receptors differ in their extracellular domain, in which IL-1R possesses Ig-like domain, whereas TLR has leucine rich repeats (LRR). The intracellular region is the same, which contains Toll/IL-1R (TIR) domain indicating the high level signaling similarity between the receptors [2]

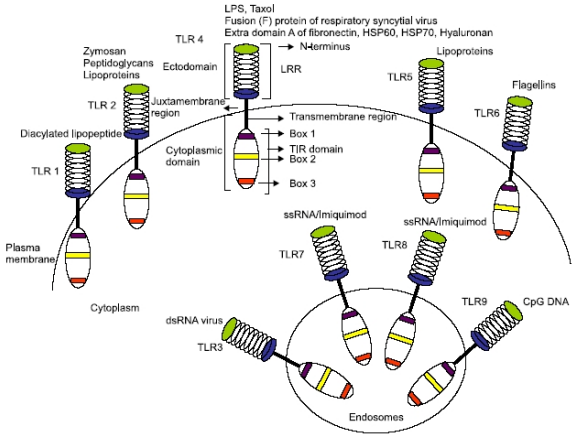

Fig. 3. The localization of TLRs in the cell [2]

2.4 TLR Ligands

When bacteria enter hosts, pathogen recognition receptors elicit signal transduction events leading to both innate and adaptive immunity. Conserved patterns unique to the microbial surface are called pathogen associated molecular patterns (PAMPs). These surface patterns allow the cell to distinguish dangerous non-self molecules from self-molecules. These PAMPs are called microorganism-associated molecular patterns (MAMPs), because they are found not only in pathogenic, but also in nonpathogenic commensal microorganisms [1]. The defense against invading micro-organisms is triggered by the ability of TLRs to recognize structurally conserved MAMPs of microbial origin. Such specific microbial products include LPS, bacterial lipoproteins, peptidoglycan, bacterial DNA, and viral nucleic acids. Activation of these conserved motifs initiates an inflammatory cascade that attempts to clear the offending pathogen and set in motion a specific immune response [4].

Table 2. TLRs and their ligands [2]

TLRs	Adapters	Location	Species	Ligands
TLR1	MyD88/Mal	Cell surface	Human/Mouse	PAM3CSK4
TLR2	MyD88/Mal	Cell surface	Human/Mouse	PAM2CSK4, MALP2, LTA, ZYM
TLR3	TRIF	Cell compartment	Human/Mouse	dsRNA, Poly-IC, viral RNA, siRNA, endogenous mRNA
TLR4	MyD88/Mal/TRIP/TRAM	Cell surface	Human/Mouse	LPS, MMTV, VSV-G, Taxol, F protein, Fibronectin, HSP60, HSP70, Hyaluronan
TLR5	MyD88	Cell surface	Human/Mouse	Flagellins
TLR6	MyD88/Mal	Cell surface	Human/Mouse	MALP2, LTA, Zym
TLR7	MyD88	Cell compartment	Human/Mouse	ssRNA, IAQ (R848)
TLR8	MyD88	Cell compartment	Human/Mouse	ssRNA, IAQ (R848)
TLR9	MyD88	Cell compartment	Human/Mouse	CpG-ODN
TLR10	Unknown	Cell surface	Human	Unknown
TLR11	MyD88	Cell surface	Mouse	Profilin
TLR12	Unknown	Unknown	Mouse	Unknown
TLR13	Unknown	Unknown	Mouse	Unknown

CpG-ODN, synthetic oligodeoxyribonuclotides containing CpG motifs; IAQ, imidazoquinolines, including resiquimod and imiquimod; LTA, lipoteichoic acid; MAL, MyD88 adapter-like; MALP2, macrophage-activating lipopeptide 2; MMTV, mouse mammary tumor virus; PAM3CSK4, synthetic triacylated lipopeptide Pam_3Cys-SKKKK × 3 HCl; PAM2CSK4, synthetic diacylated lipopeptide Pam_2Cys-SKKKK × 3 TFA; Poly-IC, polyinosinic-polycytidylic acid; ssRNA, single stranded RNA; VSV-G, vesicular somatitis virus G protein; ZYM, zymosan.

Representative MAMPs are bacterial cell-wall components. The cell walls of Gram-negative bacteria contain LPS, an immune adjuvant identified first as a TLR ligand. LPS is recognized by TLR4 and a TLR4-associated soluble factor, MD-2, derived from the host [8].

TLR2 responds not only to mycobacteria, but also to the yeast cell wall component zymosan, and Gram-positive bacteria. There are two proposed mechanisms that could explain why TLR2 recognizes a wide spectrum of microbial components. The first is that TLR2 forms heterophilic dimers with other TLRs such as TLR1 and TLR6, both of which are structurally related to TLR2. The second involves recognition of fungal-derived components by TLR2 [3]. TLR1 and TLR6 functionally associate with TLR2 and discriminate between diacyl or triacyllipopeptides. TLR6 associates with TLR2 and recognizes lipoproteins from mycoplasma.

The expression of human TLR3 in the double-stranded RNA (dsRNA)-non-responsive cell line 293 confers enhanced activation of NF-κB in response to dsRNA. TLR3 is implicated in the recognition of dsRNA and viruses [3].

TLR4 is an essential receptor for LPS recognition. Furthermore, TLR4 has been shown to be involved in the recognition of endogenous ligands, such as heat shock proteins (HSP60 and HSP70), the extra domain A of fibronectins, oligosaccharides of hyaluronic acid, heparan sulfate and fibrinogen [3].

TLR5 mediates the induction of immune response by bacterial flagellins [2].

Recent studies have showed that ssRNA is the natural ligand for TLR7/8.

TLR9 has been shown to recognize unmethylated bacterial CpG DNA.

TLR11 is involved in the recognition of uropathogenic bacteria. The natural ligands for TLRs 10, 12 and 13 are still not known.

3. TIR DOMAIN-CONTAINING ADAPTER MOLECULES IN TLR SIGNALING

For the last decade, the PRRs conversion of information from the recognition of a pathogen into an appropriate cellular response has been the subject of intense investigation. The signaling pathways activated by TLRs are broadly classified into MyD88-dependent and independent pathways. MyD88 is the universal adapter protein recruited by all TLRs except TLR3 [2, 3].

The ability of a TLR to tailor inflammatory response, specific for individual ligands, has directed research into the cytosolic subfamily of adapter molecules. These molecules orchestrate and fractionate these downstream signalling events. There are currently five cytosolic TIR-containing proteins (MyD88, Mal, TRIF, TRAM, SARM) that are thought to play a crucial role in specificity of individual TLR-mediated signalling pathways. Most TLR members differentially utilise many of these signaling components [4].

3.1 MyD88 is the Primary adapter for microbial signaling

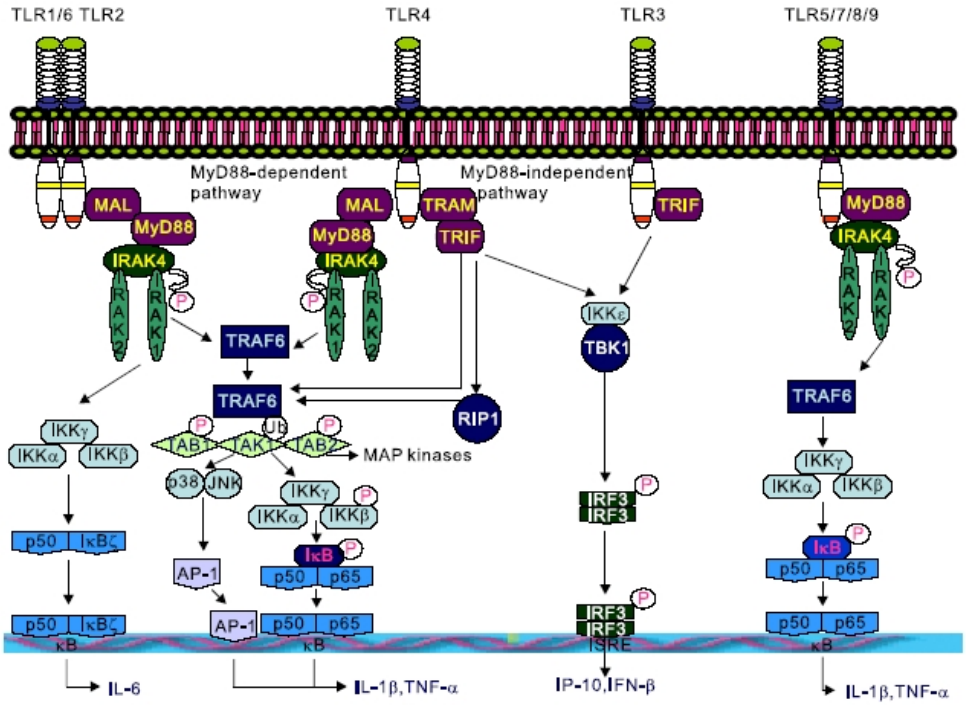

Fig. 4. Signaling mediated by TLR is broadly classified as MyD88-dependent and independent pathways [2]

The most widely utilized adapter molecule in TLRs is myeloid differentiation factor 88 (MyD88). MyD88 (a 296 amino acid protein) was originally identified as a novel myeloid differentiation primary response gene in M1 monoleukemic cell lines that show IL-6-mediated myeloid cell differentiation. It is regarded as a common adapter protein that signals by using all members of the TLR and IL-1 receptor type I (IL-1R) superfamily (Fig. 4). TLRs and receptors of the IL-1 family interact with MyD88 via their respective TIR domains to activate the Rel transcription factor NF-κB. Homophilic associations involving two well-

defined structural domains of MyD88 mediate these interactions. The carboxy-terminal TIR domain interacts with the cognate domains in the cytoplasmic tails of the TLRs (residues 155–196). The aminoterminal death domain mediates interaction with the corresponding domain of IL-1R-associated protein kinase (IRAK), a member of a family of serine-threonine kinases [4].

3.2 MAL/TIRAP Specificity in TLR Signaling

MyD88 adapter-like (Mal) or TIR domain-containing adapter protein (TIRAP) was simultaneously described as the second TIR-containing adapter protein capable of mediating NF-kB activation and responsible for differential signaling by TLR4 [9, 10]. The primary function of MAL in TLR signaling seems to control the recruitment of MyD88 to TLR4 [2].

3.3 TRIF/TICAM-1

Further database searches identified a third TIR-containing putative adaptor molecule termed TIR domain-containing adapter inducing interferon-**β** (TRIF, also known as TICAM-1, 712 amino acids). TRIF is an adapter for TLR3 and TLR4, and is associated with the MyD88-independent cascade [2]. The fact that TRIF preferentially activates the IFN-β promoter suggests the involvement of TRIF in the MyD88- independent pathway of TLR3 and TLR4 signaling [4].

3.4 TRAM/TIRP/TICAM-2

TRIF-related adapter molecule (TRAM, also known as TIRP/TICAM-2, 235 amino acids) was identified as a small TIR domain-containing protein. TRAM is placed upstream of TRIF in the induction of IFN-β through the MyD88-independent pathway. Like TRIF, TRAM activates both IRF-3 and NF-**κ**B, which coordinates the transcription of IFN-**β** and chemokines. The role of TRAM in LPS signaling appears to be as a bridging adapter connecting TLR4 and TRIF.

3.5 SARM

A fifth adapter molecule termed sterile and HEAT–Armadillo motifs (SARM, 690 amino acids) was described as containing a C-terminus TIR domain. Its role as either a positive or negative signaling component in TLR signaling is still to be fully elucidated [11].

4. KINASES INVOLVED IN SIGNALING FROM ADAPTERS TO TRANSCRIPTION FACTORS

4.1 Downstream TLR Signaling by Adapters is Mediated by the IRAK Family

So far, four IRAKs have been identified: IRAK1, IRAK2, IRAK4 and IRAKM. IRAK1 and IRAK4 possess intrinsic serine/threonine protein kinase activities, whereas IRAK2 and IRAKM lack this activity, that may negatively regulate TLR mediated signaling [2].

4.2 TRAF6 is the Central Activator of MAPK during Microbial Infection

TRAF6 is the activator of the canonical NF-κB pathway. TRAF6 is ubiquitinated at K63 chains. This K63 polyubiquitinated TRAF6 mediates the activation of the next component in the pathway, which is most likely TGF-β activated kinase-1 (TAK1).

5. TRANSCRIPTION FACTORS ACTIVATED BY TLR ENGAGEMENT

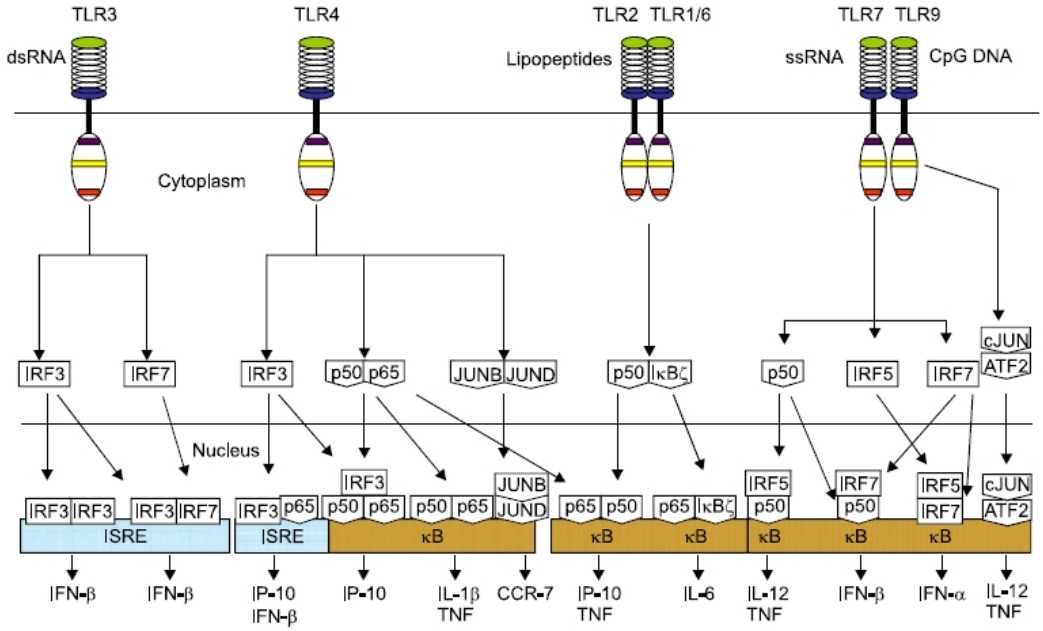

Fig. 5. Interaction of transcription factors leading to a highly specific gene expression upon TLR stimulation. ISRE, interferon stimulatory response element; κB, κB site [2]

5.1 NF-κB as Double Edged Sword

NF-κB is the major transcription factor that functions on TLR signaling to control/elicit inflammation. NF-κB was first described as a B cell specific transcription factor that binds the κB site in the Igκ light chain enhancer [12]. NF-κB has often been called a 'central mediator of the immune response'. MAL-MyD88 and TRAM-TRIF pathways stimulate NF-κB activation albeit with different kinetics. NF-κB activity was found to be inducible in all cell types (Fig.5). It is now known that the members of the NF-κB/Rel family regulate many genes involved in immune and inflammatory responses [2].

5.2 Activating Protein-1 (AP1)

The activation of TAK1 during TLR signaling results in the activation of MAPKs, including JNK/p38, leading to the activation of AP-1, which together with NF-κB governs the production of inflammatory cytokines and chemokines. Activation of these JNK/p38 cascades is associated with selective activation of different AP-1 subunits and transcription factors interacting with AP-1 [2].

5.3 IRFs are Novel Regulators of the TLR Pathway

IRF3 and IRF7 have recently been identified as the master regulators of type 1 IFN activation. However, there are many unanswered questions about their biology, structure, function, and crosstalk with other important transcription factors. A recent major breakthrough implicated two IκB kinase-related kinases, IKKε (also known as IKKi) and TBK1 (TANK-binding kinase 1), in the IRF3 pathway. Both IKKε and TBK1 directly phosphorylate IRF3, a property not shared by either IKKα or IKKβ [2].

IRF-5 also colocalizes and associates with MyD88 and TRAF6. In TLR7/9 signaling IRF-5 was required for the induction of inflammatory cytokines, but not for type I IFN induction. MyD88 also interacts with another IRF, IRF-4. IRF-4 negatively regulates the signaling pathway. IRF-4 deficiency does not affect the ability of TLR7/9-stimulated PDCs to secrete IFN-a, but can cause overproduction of inflammatory cytokines. This was accompanied by enhanced activation of NF-**κ**B and MAPKs [1].

6. ADJUVANT EFFECTS OF TLRs

The main functions of TLRs are induction of inflammation and establishment of adaptive immunity. TLR signaling can induce robust production of inflammatory cytokines, including IL-6 and TNF-**α**. These cytokines then activate surrounding cells to produce chemokines or adhesion molecules, thereby recruiting various inflammatory cells into the infection sites. Recruited macrophages or neutrophils are activated and ingest invading pathogens through internalizing PRRs. Subsequently, those cells kill them by producing nitric oxide, reactive oxygen species, or defensins [1].

TLR-activated APCs enhance expression of surface molecules and activate T cells, together with antigen presentation. CD41 T cells differentiate into TH1 or TH2 cells. TH1 cells produce IFN-**γ** and mediate antiviral or antibacterial immunity. TH2 cells secrete IL-4, IL-13, or both and are involved in allergic reactions or immunity against helminths. Most TLR ligands, however, stimulate APCs to produce TH1-inducing cytokines, such as IL-12 and IL-18, to support TH1-skewed immune responses.

Damage-associated molecular patterns (DAMPs) include endogenous intracellular molecules released by activated or necrotic cells and extracellular matrix (ECM) molecules that are upregulated upon injury or degraded following tissue damage. DAMP activation of TLR induces inflammatory gene expression to mediate tissue repair. However, DAMPs have also been implicated in diseases where excessive inflammation plays a key role in pathogenesis, including rheumatoid arthritis (RA), cancer and atherosclerosis [13]. TLR signalling is also crucial for inflammasome activation (signal 1) as another important PRR platform [14].The species- and organ-specific expression of PRR like the TLRs [15], the NLRs/RLR [16] and the C-type lectins [17]. IRF4 deficiency was also shown to have a role in systemic autoimmunity [18].

7. TOLL-LIKE RECEPTORS AND THEIR POTENTIAL ROLES IN KIDNEY DISEASE

TLR are an emerging family of receptors that recognize pathogen-associated molecular patterns and promote the activation of leukocytes and intrinsic renal cells. Tubular epithelial cells are among the non-immune cells that express TLR1, -2, -3, -4, and -6, suggesting that

these TLR might contribute to the activation of immune responses in tubulointerstitial injury (*e.g.*, bacterial pyelonephritis, sepsis, and transplant nephropathy). In addition, TLR9 has been shown to be involved in antigen-induced immune complex glomerulonephritis and lupus nephritis by regulating humoral and cellular immune responses. TLR are evolutionary conserved regulators of innate and adaptive immune responses. It is likely that TLR are involved in many if not all types of renal inflammation [19].

8. FUTURE PROSPECTS

We now know that innate immunity plays an important role in the initiation of an immune response that follows the activation of antigen-specific acquired immunity. A complete understanding of the mechanisms of innate immunity will be helpful for the future development of innovative therapies for the treatment of infectious diseases, cancer, allergies, and renal diseases.

CONSENT

In this paper, only published data from the literature were used for description. Thus, a statement of patient consent is not applicable for this paper.

ETHICAL APPROVAL

Since data from the literature, only, were used for description, ethical approval is not applicable to this paper. This study is not against the public interest. All authors hereby declare that all description have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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