Review paper

The role of Toll-like receptors in health and disease – short review

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Abstract

Toll-like receptors (TLRs) are a group of proteins involved in recognition of highly conserved microbial structures and endogenous molecules released during tissue damage, which makes them key players in innate immunity. By activating nuclear factor κB (NFκB), TLRs are also involved in several physiological processes including angiogenesis, apoptosis, and tissue repair. As shown in recent publications, disorder of TLRs signaling pathways as well as abnormalities in their expression may underlie various diseases, such as sepsis, life-threatening infections, tumors. This paper reviews the role of TLRs in innate immune response and consequences of disturbances in their functioning.

Key words: *Toll-like receptors, TLR, innate immunity.*

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Introduction

One of the mechanisms used in defense against pathogenic microorganisms are receptors belonging to Toll-like family. With the ability to recognize highly conserved microbial structures called pathogen-associated molecular patterns (PAMPs), Toll-like receptors (TLRs) are the first line of defense against bacteria, viruses and yeast. Stimulation of TLR may also occur as a result of recognition of endogenous molecules released during tissue breakdown, called danger-associated molecular patterns (DAMPs) or alarmins [1].

To date, 13 proteins belonging to TLRs family have been identified, of which 11 are expressed on human cells (TLR 1-11) (Table 1).

Presence of TLRs was demonstrated on cells involved in innate (primary) immune response – monocytes, macrophages, neutrophils, dendritic and mast cells. They are also expressed by T and B lymphocytes as part of acquired immunity [2, 3]. Furthermore, TLRs are present on epithelial cells, including intestinal, where on the one hand are involved in maintaining of peripheral tolerance to dietary antigens and commensal bacteria, on the other are involved in the inflammatory response following invasion of pathogens [4]. Due to presence in both the cell membrane (TLR1, TLR2, TLR4, TLR5, TLR6, TLR11) and the other cellular compartments (TLR3, TLR7, TLR8, TLR9), TLRs protect body against wide spectrum of pathogens. Toll-like receptors which detect structural elements derived from bacteria are located on the cell surface, whereas TLRs that recognize viral and bacterial nucleic acids are present in endosomal vesicles [5]. Stimulation of TLRs launches cascade of reactions that leads to production of proinflammatory cytokines, including interferon-α (IFN-α), tumor necrosis factor α (TNF-α), interleukins: IL-1, IL-6, IL-4, IL-8, IL-12, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) and chemokines: CCL2, CCL3 and CCL4 [6].

By activating nuclear factor κB (NFκB), TLRs are involved in several physiological processes including apoptosis, angiogenesis and tissue repair [6-8]. In experimental animals, deprivation of the gene encoding TLR3 protein causes impairment of wound healing (decreased neovascularization, re-epithelialization and granulation formation). Furthermore, in TLR3 (–/–) deficient mouse, disorder of macrophages and neutrophils recruitment as well as decreased expression of chemokins in wound were observed, which points to the essential role of TLR3 in process of wound healing [9].

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Table 1. Short characteristic of TLRs according to [2]

Receptor	Location	Cells	PAMP	DAMP
TLR1	cell membrane	B cells	lipoproteines	
TLR2	cell membrane	monocytes dendritic cells neutrophils	peptidoglykan lipoteichoic acid lipoproteines/lipopeptides	HSP HMGB1 hialuronic acid
TLR3	endosomes	dendritic cells	virus dsRNA	endogenous RNA
TLR4	cell membrane	leukocytes	lipopolisaccharide (LPS) HSP	HSP fibrinogen fibronektin
TLR5	cell membrane	dendritic cells monocytes	flagelline	
TLR6	cell membrane	makrophages dendritic cells	lipoteichoic acid zymosan	
TLR7/8	endosomes	dendritic cells makrophages	virus ssRNA	endogenous RNA
TLR9	endosomes	monocytes dendritic cells	unmetylated CpG	endogenous DNA
TLR10	cell membrane	B cells dendritic cells	unknown	unknown
TLR11	cell membrane		profilin-like protein	

Toll-like receptors structure and signaling pathway

Toll-like receptors belong to the family of type I transmembrane proteins. Their structure consist of three signal domains: extracellular, transmembrane and intracellular [10]. Extracellular domain contains 16-28 repeating leucine-rich fragments and is responsible for ligands identifying [11]. Transmembrane and intracellular domains have a structure homologous with IL-1 receptor (IL-1R) and IL-18 receptor (IL-18R) [12]. Upon stimulation with ligand, TLR undergoes dimerization followed by the association of the adaptor proteins with the TLR intracellular TIR domain (Toll/IL-1 receptor), which initiates a signaling pathway [13]. Intracellular TIR domain made up of about 200 amino acids is present also in adapter proteins MyD88 (myeloid differentiation primary-response protein-88) and TRIF (TIR domain-containing adapter inducing IFN-β). Specific interplay between TLR's TIR domain of and the TIR domain of adapter protein enables their interaction [14]. Toll-like receptors signaling can occur in MyD88 – dependent or independent manner (Fig. 1) [2, 15].

MyD88-dependent and independent pathway

After recognizing a ligand, most proteins belonging to the TLR family, except TLR3, recruit adapter protein MyD88 thereby initiating MyD88-dependent signaling pathway. Receptors TLR5, TLR7, TLR9, TLR11 recruit MyD88 protein directly, while TLR1, TLR2, TLR4, and TLR6 require presence of additional protein – TIRAP (TIR domain-containing adaptor protein), acting as adapter between the TLR and MyD88 TIR domains. Next steps of signaling pathway include recruitment of IRAK kinases (interleukin-1 receptor-associated kinase-1), activation of TRAF6 protein (tumour necrosis factor-receptor-associated factor-6), TAK1 (TGF-β-activated kinase-1) activation of the transcription factor NFκB, MAP kinase (mitogen-activated protein kinases), p38, and JNK (c-Jun kinase). Stimulation of TLR4 can activate IRF3 (interferon regulatory factor-3), resulting in production of interferon-β (IFN-β) involved in antiviral immunity. Stimulation of TLR3 initiates MyD88 – independent, TRIF-dependent signaling pathway. Interaction between C-terminal fragment of TRIF and RIP1 (receptor – interacting protein 1) or *N*-terminal fragment of TRIF and TRAF6 results in activation of the transcription factor IRF-3 [2, 15, 16].

Toll-like receptors signaling regulation

In order to avoid excessive inflammatory response associated with stimulation of TLRs, their signaling pathway is regulated negatively. Signaling cascade can be blocked either at the receptor level or the later stages of the pathway. One of the proteins that negatively regulate TLR signaling pathway is transmembrane protein SIGIRR [single immunoglobulin IL-IR-related molecule (TIR-8)] which has the capacity to inhibit inflammatory response associated with TLR4 and TLR9 [17]. Single immunoglobulin

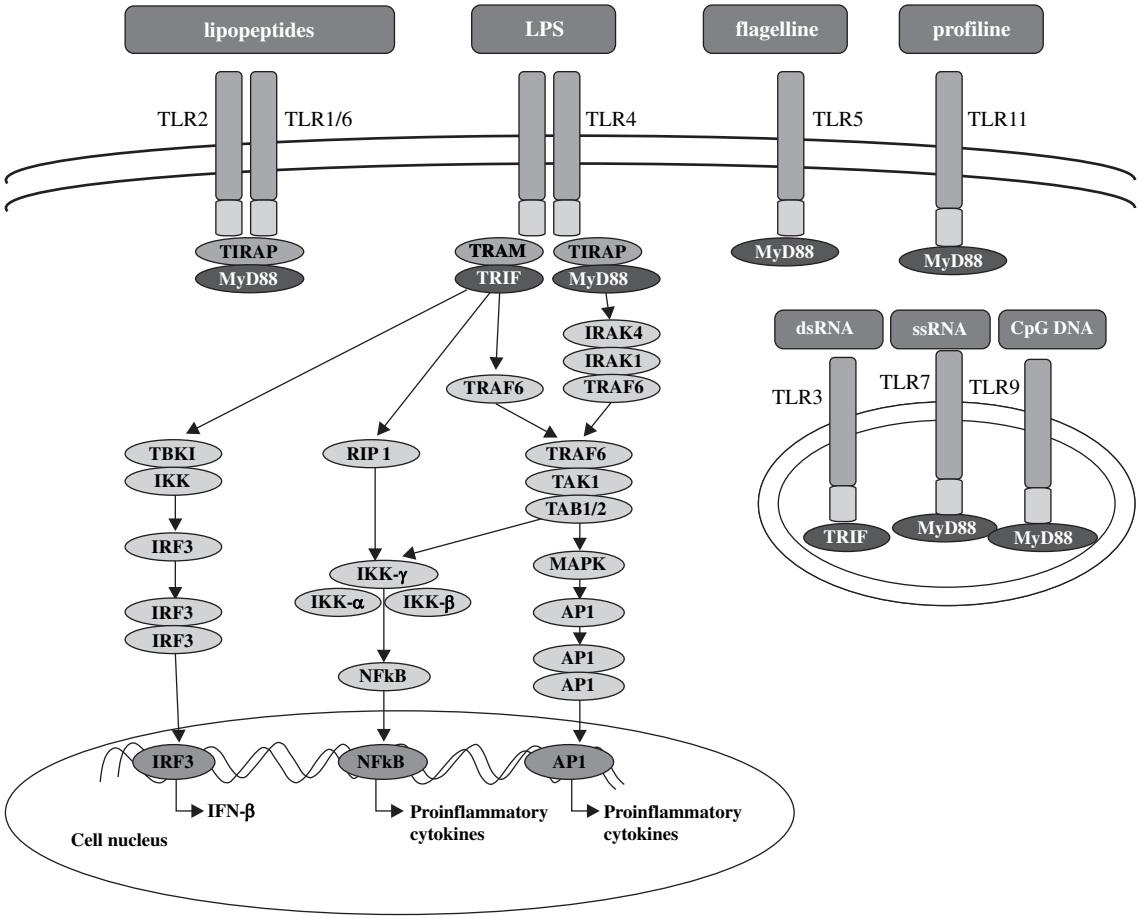

Fig. 1. TLR signaling pathway according to [2, 15]

TIRAP – TIR domain-containing adapter protein; TRAM – translocating chain associating membrane protein; MyD88 – myeloid differentiation primary-response protein-88; TRIF – TIR domain-containing adapter inducing IFN-β; IRAK1 – interleukin-1 receptor-associated kinase-1; IRAK4 – interleukin-1 receptor-associated kinase-4; TRAF6 – tumour necrosis factor-receptor – associated factor-6; TAK1 – TGF-β-activated kinase-1; MAP – mitogen-activated protein kinases; TBK1 – TANK binding kinase 1; RIP1 – receptor-interacting protein 1; TAB1/2 – TAK1-binding protein 1/2; IRF3 – interferon regulatory factor 3; NFκB – nuclear factor-κB; AP1 – activator protein 1

IL-IR-related molecule (SIGIRR) is expressed by intestinal epithelial cells, where it is involved in regulating the immune response. A study of colonic mucosal biopsies obtained from patients with ulcerative colitis showed that expression of SIGIRR is significantly higher in inactive mucosa as compared with active. The authors conclude that SIGIRR, as a negative regulator of TLR4 plays significant role in maintaining innate immunity of gut, and undergoes down regulation during inflammation [18].

The intensity and duration of TLR4 and TLR9 activation may also be regulated by Triad3A. It was shown that this protein promotes ubiquitination of TLR4 and TLR9, thereby leading to their degradation [19].

Another protein preventing abnormal excessive immune response associated with stimulation of TLRs is SOCS1 protein belonging to the SOCS family (suppressor of cytokine signaling). Study performed in mice lacking SOCS1 shown that negative regulation of TLR signaling by this protein is essential for the prevention of liver diseases such as hepatitis, cirrhosis and cancer [20]. Studies conducted in patients with primary hepatocellular carcinoma showed frequent methylation of CpG islands of SOCS1 corresponding with its transcription silencing, which may indicate the protective role of this protein [21].

Toll-like receptors signaling and disease

Disorder of TLRs signaling pathways may underlie various diseases. Deficiency of TLRs or proteins involved in their signaling pathway results in increased susceptibility to infection. Von Bernuth *et al*. [22] found that people with MyD88 deficiency are in greater risk of pyogenic bacteria infections. Picard *et al.* [23] analyzed the clinical status of 48 patients with inherited autorecessive deficiency of IRAK-4, and 12 patients with genetically conditioned

MyD88 deficiency. According to the authors, the clinical symptoms accompanying deficiency of these proteins are very similar. Patients in both groups had a high incidence of recurrent, life-threatening bacterial infections. This applied in particular to pneumococcal infections appearing during infancy and early childhood.

Studies of the other authors showed that abnormal expression of TLR receptors could be associated with sepsis, autoimmune diseases (lupus erythematosus, rheumatoid arthritis, type 1 diabetes), cancer and allergy [24-27].

In the course of sepsis an increase in the expression of TLR2 and TLR4 receptors on peripheral blood monocytes was observed by Tsujimoto *et al.* [24]. On the other hand Schaaf *et al.* [28] showed that the expression of these receptors among patients who died due to sepsis was significantly lower as compared with patients who survived severe infection. The investigators conclude that insufficient level of TLR activation in the course of sepsis can be associated with increased mortality in these patients.

Link between TLR signaling and autoimmune diseases has been observed in several studies. It is hypothesized that one of the factors contributing to rheumatoid arthritis is vicious circle started by microbial infection triggering activation of TLRs which results in inflammatory response. Disturbed inflammatory response leads to tissue damage and release of endogenous ligands for TLR, which further enhances reaction [29]. There are laboratory attempts to use SIGIRG as negative regulator of TLR signaling in potential therapy of rheumatoid arthritis [30]. In animal model of experimental autoimmune encephalomyelitis Miranda-Hernandez *et al.* [31] showed that targeted deletions of TLR2, TLR9 and MyD88 but not TLR1, TLR4, TLR6 partially or completely protected mice from the disease pointing to engagement of these proteins in development of encephalomyelitis.

Contribution of TLRs to progression of cancer has been an object of several studies. Toll-like receptor expression on cancer cells is believed to be a factor supporting their metastatic potential and facilitating evasion from immune surveillance [32]. Several investigations have shown that TLRs expression correlates positively with presence of metastases [33-35].

Conclusions

Toll-like receptors are the key players in recognition and defense against microbial pathogens. Their stimulation leads to activation of signaling cascade resulting in synthesis of proinflammatory cytokines and many chemokines. Since their signaling pathway include activation of transcription factor NFκB, TLRs take part in regulation of many physiological processes, such as apoptosis and tissue repair. Disorders of TLRs signaling cascade as well as deregulation of their expression may lead to serious diseases, such as sepsis, life-threatening infections, tumors. Due to the powerful effect of TLRs activation, there is a need of meticulous studies on their signaling pathway, its disorders as well as possibilities of its regulation.

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