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Toll Like Receptors Signaling Pathways as a Target for Therapeutic Interventions

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Abstract

This review summarizes the key role of Toll-Like Receptor (TLRs) molecules for igniting the immune system. Activated by a broad spectrum of pathogens, cytokines or other specific molecules, TLRs trigger innate immune responses. Published data demonstrate that the targeting and suppression of TLRs and TLR-related proteins with particular inhibitors may provide pivotal treatments for patients with cancer, asthma, sepsis, Crohn's disease and thrombosis. Many drugs that target cytokines act in the late phases of the activated pathways, after the final peptides, proteins or glycoproteins are formed in the cell environment. TLR activity occurs in the early activation of cellular pathways; consequently inhibiting them might be most beneficial in the treatment of human diseases.

Keywords

TLR; signaling pathways; therapy

HUMAN TOLL-LIKE RECEPTORS (TLRS)

The first report about Toll-Like Receptors (TLRs) was published in 1997, when the ortholog of Drosophila Toll molecule TLR4 was identified [1–3]. Prior to this time11 human TLRs and 13 TLR genes in mice had been identified [4]. All TLR - type transmembrane glycoproteins are critical for the innate immune response in mammals. They have very similar basal structure that contain extracellular fragments with 16–28 horseshoe-like shape leucine-rich repeat (LRR) modules and are responsible for binding “pathogen associated molecular patterns” (PAMPs) [5]. The features of these modules are determined by a conserved sequence pattern in the LRR modules ligand-induced dimerization of TLRs and this stabilizes the protein by protecting its hydrophobic core from solvents. Adaptor proteins trigger the intracellular TIR (Toll interleukin-1 receptor) domains and initiate signaling.

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They are followed by the transmembrane domain and intracellular signaling domain composed of about 150 amino acid residues (Fig. 1).

Classification of TLRs divides them into five subfamilies: TLR2, TLR3, TLR4, TLR5, and TLR9 [6] (Table 1). Although TLR subfamilies have a similar structure, it does not mean that they are similar in their function. The common feature of all TLRs is that they are structurally modified by posttranslational modification of N-linked glycosylation consensus sites. This modification affects receptor surface representation, trafficking, and pattern recognition (Table 2). It has been shown that gp96 (endoplasmin) is required for cell-surface expression of TLR1, TLR2, and TLR4. Placed on endoplasmatic reticulum, gp96 is necessary for proper protein folding. This chaperonin protein is required for post-translational folding of TLRs to produce functional receptors and for expression of both intracellular and cell surface TLRs. Deletion of gp96 resulted in functional deficiency in all studied TLRs [7,8]. TLR1, TLR2, TLR4, TLR5, and TLR6 are found on the plasma membrane. In contrast, TLR3, TLR7, TLR 8, and TLR9, which detect double-stranded RNA (dsRNA), single stranded RNA (ssRNA), and un-methylated DNA, mainly reside in the endoplasmic reticulum, endosomes and lysosomes [7].

TOLL LIKE RECEPTORS SIGNALING PATHWAYS

TLRs intracellular fragments are structurally similar to parts of IL-1 receptors, thus they are likely to share the same signaling pathways. To be activated some TLRs appear to require dimerization. The TLR2 receptor is an excellent example of such a heterodimer connection establishing heterodimers with TLR1 or TLR6 (Fig. 2). The signaling cascades *via* the TIR domains are mediated by specific adaptor molecules including MyD88, MAL (also known as TIRAP), TRI, and TRAM [9]. The adaptor proteins containing TIR domains and TIR-TIR interactions between receptor-receptor, receptor-adaptor, and adaptor-adaptor are critical for activation and signaling [9]. Upon ligand binding to TLRs, the adaptor molecule MyD88 is recruited to the TLR complex as a dimer. The binding of MyD88 promotes association with IL-1 receptor–associated kinase-4 (IRAK-4) and IRAK-1. TNF-α associated factor 6 (TRAF6) is recruited to IRAK-1. The IRAK-4/IRAK-1/TRAF6 complex dissociates from the receptor and then interacts with another complex consisting of TGF-β-activated kinase (TAK1), TAK1-binding protein 1 (TAB1), and TAB2. TAK1 is subsequently activated in the cytoplasm, leading to the activation of IκB kinase kinases (IKKs). IKK activation leads to the phosphorylation and degradation of IκB and the consequent release of Nuclear Factor-κB (NF-κB). Translocation of NF- κB into the nucleus activates inflammatory chemokines and cytokines. TRIF (TIR-domain-containing adaptor protein inducing interferon-β), and TRAM (TRIF-related adaptor molecule) both mediate MyD88-independent induction of interferon by activating the expression of different types of interferon-inducible genes.

Toll like receptors have ability to bind a wide spectrum of agonists. This bond initiates variety of cell responses. A wide of spectrum of TLRs may serve as biomarkers of various diseases. TLRs are expressed by many cell types including endothelium, fibroblasts, vascular smooth muscle cells, memory T-cells, regulatory T-cells, mast cells and dendritic cells. Some TLRs such as TLR2, TLR3, TLR4 and TLR6, are also detectable in cardiac myocytes. Activated TLRs are also able to mediate pro-inflammatory signaling in

pulmonary inflammatory disease. Respiratory epithelial cells are the initial site of bacterial colonization in the respiratory tract. Airway and alveolar type II epithelial cells are able to up-regulate TLR expression on the apical surface enhancing antimicrobial responses [10]. Subsequently TLR-driven responses can activate airway smooth muscle cells and neutrophils. Pharmacologic regulation of such a mechanism may be effective in the treatment of lung diseases.

It has been shown in the development of many diseases that polymorphism of TLRs may intensify the process. The association between TLR polymorphisms and infectious diseases or cancers has been reported in relation to TLR2, TLR4, TLR5 and TLR9. Other TLRs, like TLR1, TLR3, TLR6 or TLR10 seem to be very rare and are poorly understood. TLR2, TLR4, TLR6, and TLR9 polymorphisms are associated with the regulation of TLR expression and development of active tuberculosis [11]. TLR 9 gene polymorphism may be involved in the pathogenesis of Hodgkin's lymphoma through altered inflammatory responses [12]. It was also shown that TLR polymorphisms influence COPD development and severity in smokers. Single nucleotide polymorphism impaired TLR4 signaling and allowed receptor hyporesponsiveness in alveolar macrophages, epithelial cells, and peripheral blood mononuclear cells. In this manner TLR4 polymorphism dysfunction may contribute to COPD pathophysiology [13].

Data from the literature indicate that TLR-mediated recognition is mainly involved in the TH1 immune response [14–17]. The TH1 response is involved in inflammation and in fighting viruses and bacteria, and also it is also important in specific autoimmune diseases e.g. insulin-dependent diabetes mellitus. The antinflamatory action of the TH2 response counteracts the effect of the TH1 cytokines and engaged in allergy development. Allergens lack PAMPs, but some of them may be recognized by TLRs. They probably initiate adaptive immune responses by TLR-independent mechanisms. It is also possible that TH2 responses are TLR dependent, by the MyD88 independent pathway [5] (Fig. 2). Studies demonstrate that smoking during pregnancy significantly attenuates TLR-mediated immune responses. It increases the risk of the offspring's development of allergies and asthma [18,19]. Overexpression of TLR4 has been shown during atherosclerotic disease progression. This is demonstrated by clinical manifestations of atherosclerotic disease that correlate with cytokine release after TLR4 stimulation. Also TLR4 expression is significantly elevated in patients suffering from recurrent unstable angina, compared with healthy controls [20]. It has also been found that strong changes in cellular TLR responsiveness are induced by local vascular injury. TLRs, by signaling the presence of infection, can direct the adaptive immune responses against antigens of microbial origin. Immature Blood Dendritic Cells (DCs) express a full set of TLRs that actually mediate their own maturation. Mature DCs express high levels of MHC, CD80, CD86 and migrate to draining lymph nodes to present pathogen-derived antigens to naive T cells. Dendritic Cell TLRs are then able to induce expression of a number of cytokines e.g. Interleukin 12 (IL-12) [21–25]. In the presence of activating factors several mammalian TLRs increase synthesis and release of vascular endothelial growth factors (VEGF) [26].

TLR2 AS EXAMPLE OF TLR INVOLVEMENT IN A WIDE SPECTRUM OF DISEASES

Recently, TLR2, has been thought to have an impact on the development of some inflammatory diseases like acute lung injury (ALI). ALI and its more severe form, the acute respiratory distress syndrome (ARDS), derive from acute pulmonary edema and inflammation caused by trauma, sepsis, acute pancreatitis and drug overdose. The TLR2 pathway may be activated by the Toll interacting protein (Tollip) and subsequently suppress the activity of IRAK [27]. Tollip is known as a TLR regulator that is presented in a complex with IRAK-1. Upon stimulation with IL-1, the Tollip-IRAK-1 complex is recruited to the IL-1 receptor complex, allowing for IRAK-1 phosphorylation, dissociation from Tollip and TRAF6 activation. The mechanism of Tollip overexpression that leads to the inhibition of NF-βB activation is still poorly understood. [28] (Fig. 3). Negative regulation of TLR signaling by Tollip may limit the production of proinflammatory mediators during the inflammation process following an infection [29,30]. Although TLR2 shares the MyD88 signaling pathway with other TLRs, it may possess a unique signaling track. The most well known pathway is the TLR2-mediated signaling of human monocytes by Staphylococcus aureus. TLR2 is the receptor that also mediates signaling on numerous other ligands including: peptidoglycan from Gram-positive bacteria [31], bacterial lipoproteins [32–35], the mycobacterial cell wall [36–40], lipoarabinomannan [39], glycosylphosphatidylinositol (a lipid from Trypanosoma Cruzi) [41,42], yeast cell walls [43–49], atypical LPS from Leptospira interrogans [50] and Porphyromonas gingivitis [51,52]. It induces a fast and transient activation of the Rho GTPases - Rac1 and Cdc42 [53,54]. Recruitment of active Rac1 and phosphatidylinositol-3 kinase (PI3K) to the TLR2 cytosolic domain leads to activation of Akt kinase [53,54]. This allows activation and translocation of the p65 subunit of NF-κB into the cell nucleus in a process that is independent of IκB degradation (Fig. 2).

A wide spectrum of agonist activity is possible because TLR2 is supported either by TLR1 or TLR6 ability to create heterodimers [5]. Some other TLRs also create heterodimers e.g. TLR7 with TLR8. In addition TLR4 also establishes homodimers. In contrast, TLRs like TLR3, TLR5 and TL9 do not necessary require other TLRs to be activated. These differences demonstrate possible ways to regulate the activity of TLRs. Both TLR1 and TLR6 are expressed constitutively on many cell types, whereas the expression of TLR2 is regulated and seems to be restricted to antigen-presenting cells, smooth muscle cells, fibroblasts and endothelial cells [5], [20]. TLR2 is mainly known as a mediator of macrophage recognition of mycobacteria and gram-positive organisms [55]. Both TLR2 and TLR4 are associated with the inflammatory responses in the pathogenesis of atherosclerotic plaque destabilization and intimal hyperplasia after arterial injury [20,56–58]. TLR2 detects circulating, immunologically active cells, thus it could play role in innate immunity. TLR2 also has the ability to bind with the lipid scavenger receptor molecule, CD36 in response to diacylated lipoproteins [32,59–62]. Heterodimers TLR2/TLR1 and TLR2/TLR6 are pre-existing and internalize the corresponding ligand since their formation does not seem to be induced by the TLR2/1/6-binding molecules or PAMPs [63]. In contrast, interaction of TLR2/6 with CD36 is not activated unless it is induced by the ligand [32,63]. The ligand-binding pockets of TLR1 and TLR2 are located at the boundary of the central and C-

terminal domain in the convex region. The flexible loops at the domain boundaries are separated, forming crevices that are connected to large internal pockets. The pockets of TLR1 and TLR2 are bridged by the bounded ligand and therefore form a long continuous hydrophobic pocket [64]. Due to the molecules diversity, it seems unlikely that TLR2 has the capability to react with all agonists to the same degree [6]. Nevertheless, TLR2 involvement in a large number of inflammatory diseases has been reported: rheumatoid arthritis, type I diabetes, inflammatory bowel disease, ischemia/reperfusion injury, vascular injury and atherosclerosis. There are also some suggestions that this genetic variation in TLR2 is a major determinant of susceptibility to asthma and allergies [3]. Genetic variations in TLR2 are responsible for an observed protection of farmers' children from allergy and asthma [18]. Prenatal exposure to farm stables upregulates TLR2 expression in neonatal cells [19]. TLR2 and TLR4 activation has been shown to reduce apoptosis of cardiac myocytes, which suggests that TLR signaling may be important in the development of myocardial diseases [3]. Some data link alcohol with upregulation of TLR2 followed by the production of an inflammatory response in the airway epithelium after a bacterial challenge. Cells were shown to double their IL-8 release when exposed to low stimulation levels of the TLR2 ligand. It has been shown that IL-8 secretion is increased by oxidant stress and conversely, IL-8, by causing recruitment of inflammatory cells, which induce a further increase in oxidant stress mediators, making it a key parameter in localized inflammation [65]. The TLR2 pathway was also noted to increase in bronchitis after alcohol consumption. Alcohol seems to prime airway cells and enhance airway inflammation inevitably leading to severe airway disease. The intensity of response with alcohol is dependent on the alcohol dose and upon the cell type [66]. Takagi *et al*. showed that the alleles and genotypes that included short GT repeats in intron 2 of the TLR2 gene were associated with acute pancreatitis in the Japanese population [67]. During carcinogenesis the nuclear factor–κB signaling pathway is activated [68]. Yim *et al*. reported that alleles with high and low numbers of GT repeats had greater promoter activities than those with medium numbers of repeats when stimulated with interferon [69]. However, the same investigators reported that shorter GT repeats were associated with weaker promoter activities and lower TLR2 expression on CD14 positive peripheral blood mononuclear cells. It was shown that uric acid crystals, product of purines catabolism, acting through TLR2 or TLR4 initiate inflammation in tumors [70]. Elevated levels of uric acid are produced by injured tissue and at high concentration uric acid precipitates and forms crystals causing inflammation [71,72]. Recent studies show that uric acid, acting on TLR2 and TLR4, is responsible for inflammation in lungs [71]. TLR2 and TLR4 acting through CD14 regulate uptake of uric acid crystals by alveolar macrophages [72]. A published study demonstrated that combined action of TLR2 and TLR4 is required for optimal inflammation in response to uric acid crystals during lung injury [71].

CLINICAL SIGNIFICANCE OF TLRS - HOW WE MIGHT TARGET TLRS THERAPEUTICALLY

Structurally, the domains of the TLRs may potentially be used as drug targets (Fig. 4). The ectodomain, which carries the site of ligand recognition, is unique to each TLR. Hsp10 treated cells reduced lipopolysaccharide (LPS)-induced NF-κB activation, regulated by TLRs and the secretion of several inflammatory mediators. Hsp10 treatment also delayed

mortality [73]. Hsp10 acting through TLR4 has been shown to reduce symptoms of rheumatoid arthritis [74]. Literature also supports the possibility of limiting TLR2 signaling by blocking TLR2 with a neutralizing antibody or antisense oligonucleotides, or to regulate them by small-molecule inhibitors [75–78]. Rapid action by these inhibitors may be a possible tool in fighting sepsis or inflammatory disease [79,80]. For example TLR2 knockout mice are hyporesponsive to Gram-positive bacteria cell wall components [31]. Thus targeting TLRs could thus be important in the development of disease treatment. Many of the drugs, that target cytokines, act in the late phase - at the end of activated pathways. This may make treatment less effective, because final pathogenic peptides, proteins or glycoproteins are already in the cell and deregulating the cell environment. TLRs activity occurs in the early phase, thus inhibition of TLRs in the early phase might be more effective in activating or inhibiting the cascade that leads to inflammation and disease.

Some medications targeting TLRs have already been approved by the Food and Drug Administration (FDA) and even more are being tested in numerous clinical trials [81–87]. Imiquimod, approved in 1997 acts as an immune response modifier through TLR7 [88–92]. It leads cells to secret cytokines, IFN-α, IL-6 and TNF-α. This medication treats certain diseases of the skin, superficial malignant melanomas, and genital warts [93]. In 2005 the successful phase ll clinical trial results of E5564 (generic name eritoran), a drug candidate for treating severe sepsis, was reported [94–97]. The results showed a certain reduction in the mortality rate of the group with high-dose eritoran. Specifically, the rate was reduced by 6.4% compared to the placebo group. E5564, is a Lipid A derivative, which by antagonizing TLR4, blocks the receptor signal transduction. Subsequently it inhibits the release of the inflammatory cytokines IL-1 and TNF-α, and suppresses the development of severe sepsis [96–98]. TLRs are also known to be directly related to cancer development and progression. TLRs heterogeneity allows different regulation and expression of the genes related to inflammation and immunity. Understanding and proper targeting of TLRs could result in novel treatments for cancers [99]. Recently, it has been shown that carbohydrate-based cancer vaccines could act by an artificial linker to a carrier protein [100,101]. One of the components of the three component vaccine is a TLR2 activator faciliating incorporation of TLR activators. It induces cytokines necessary for maturation of immune cells, leading to a strong antigenic response against tumor-associated glycopeptides antigens. Improved cancer cell recognition was observed when the TLR2 epitope was covalently attached to the glycopeptide on the lymphocyte surface [100,101]. The search for an efficient cancer vaccine focuses on the development of a link between two components: an antigen and an adjuvant. A TLR activator could be a part of a designed vaccine, by determination of maturation of dendritic cells, natural killer cell activation for cellular immune response and an antibody necessary for humoral immune response. It could have both therapeutic and preventive effects in cancer treatment. It was shown that cancer treated with peptide chimeras generated from a TLR2 agonist (small lipopeptides) and anti-tumour antibodies show a decrease in development [102]. Pretreatment of tumor cells with TLR ligands resulted in an increased production of granzymes and an enhanced killing capacity of T-cell lines [103]. It was also shown that TLR5 ligation with flagellin can convert dendritic cells from tolerogenic into activating antigen-presenting cells providing material for cancer immunotherapy [104]. The targeting of TLR9 reveals a possible treatment an complex

pathology in diseases like asthma [105]. G oligonucleotides (CpG–ODN, resembling bacterial DNA) have been shown to activate TLR-9 on leukocytes B and dendritic cells, resulting in the induction of Th1-immune response and an interruption of mast cell signaling and the blocking of IgE mediated pathways [106]. IMO-2055, a TLR-9 agonist, is under clinical evaluation in oncology patients. Another TLR-9 agonist IMO-2125 induces high and sustained levels of IFN and is being evaluated in Hepatitis C infected human subjects [107].

Another therapeutic potential of TLRs may be to target the inflammatory lung manifestations of cystic fibrosis [108]. For example, TLR3 affects osteoclastogenic activity and thus may well have an impact on rheumatoid arthritis (RA) development or progression [109]. Expression of TLR3 is much higher in the RA synovium than in osteoarthritis synovium [110,111]. TLR3 may also affect human monocyte osteoclast differentiation Targeting the TLR3 pathway with for example Polyinosine-polycytidylic acid (poly(I:C) – a synthetic analog of double-stranded RNA, could block inflammatory bone destruction in RA or development of cancer [112] [109].

TLRs e.g. TLR2 and TLR4 are also involved in primarily non-immune-related diseases like hepatic ischemia reperfusion injury (IRI) [113–115]. TLRs activation of Kupffer cells trigger pro-inflammatory signaling responses that lead to liver IRI. This pathway offers another potential for cure and signal modulation could have a beneficial effect in patients with liver IRI [116]. Very recently it was shown that ischemia reperfusion injury following kidney transplantation in humans involves signaling through TLR4 expressed in donor kidney cells [117]. As Kruger *et al.* pointed in their paper, targeting TLR signaling by specific inhibitors may have several potential implications beyond protection against ischemia and reperfusion injury and increase the success of tolerogeneic protocols in the long-term maintenance of graft function and survival [117].

There are several reports that TLRs are highly expressed in endothelium and heart muscle cells, suggesting a functional importance of TLRs in the cardiovascular system [86,118,119]. Acting with specific inhibitors through TLRs could have a therapeutic effect in patients diagnosed with cardiac disorders including atherosclerosis, heart failure, myocarditis, septic myocardial dysfunction and diabetic antipathies [120]. Human studies have established that unstable angina and acute myocardial infarction are associated with enhanced expression and signaling events downstream from human TLR4 in circulating monocytes [3,121]. In monocytes TLR4 has been shown to be activated in acute heart failure after myocardial infarction [122]. Interestingly, therapeutic use of TLR4 inhibition is suggested in thrombosis, transplant atherosclerosis, restenosis after angioplasty with stenting, and in vein-graft disease after bypass surgery [123]. The vascular delivery of TLR4 or MyD88 inhibitors can be accomplished by coating them with compound inhibitors, and by administering recombinant viral vectors that deliver genes expressing antisense TLR4 RNA, or by small-molecule antagonists [123]. Other signaling inhibitors including antiapoptotic protein A20, agonistic lipid A and its analogues, inhibitors of IkB2, salicylate and parthenolide, NF-B kinases inhibitor PS-1145, inhibitors of p38, JNK, anti-TLR, anti-MyD88 antibodies, are able to target the TIR domain of the TLR4 or the MyD88 [79,124,125].

TLR2 and TLR1 expression is elevated in human atherosclerotic lesions and injection of exogenous TLR2/1 agonist exacerbated atherosclerosis [126]. Accumulation and upregulation of TLR2, TLR4 and its signaling pathway products, have been reported in myocarditis. Recent studies demonstrate that it is possible to develop effective therapies with a cardioprotective effect, which acts through T regulatory cells *via* the TLR5 and TLR9 [120,127,128]. Others compounds such as CBLB-501 and CBLB-502, recombinant flagellin proteins have been isolated from activated NFkB and act *via* TLR5 for protection against tissue injury in conditions involving high levels of apoptosis caused by radiotherapy, other radiation exposure and hypoxia. TLRs can also activate vascular damage in diabetic patients, as well as affect other organs [18,129–133]. Ligation of TLRs allows for ongoing inflammatory processes that run independently from the glucose level, even when homeostasis is restored. Some anti-inflammatory agents, TLR4, TLR2, TLR3, and MyD88 inhibitors as well as statins and thiazolidines have been suggested to treat diabetes and obesity-associated cardiovascular disorders [134]. Interference with TLR signaling by these agents may turn off the inflammatory process that triggers diabetic complications or reduce the extent to which complications occur. TLR4 has been shown to participate in the regulation of energy balance and insulin resistance in response to changes in the nutritional environment. Certain dietary lipids activate TLR4 and can promote insulin resistance influencing the development of type 2 diabetes. An interaction with TLR4 or MyD88 signaling pathways was demonstrated during developmental research for effective medications like fluvastatin, simvastatin, and atorvastatin. TLR1, TLR2, TLR3, and TLR7 seem to have a double nature: they play a beneficial role in host defense but may also trigger a strong autoimmune response that can lead to diabetes or other autoimmune diseases. These findings demonstrate that TLR pathways are involved in mediation of islet inflammation. This link between TLR upregulation and autoimmunity is another area of potential new therapeutic modalities in the pathways involving TLR agonists, especially as vaccine adjuvants [135].

Developmental efforts are focused primarily on compounds targeting specific TLRs such as TLR4, TLR2, TLR3, TLR5, TLR7 and TLR9. Because of multiple molecular links between chronic infection, inflammation, cardiovascular disease and TLRs, modulating just one receptor might not produce a complete immune response. Effective treatment and prevention may require an integrated approach that utilizes a combination of strategies to target the underlying inflammatory processes. The TLR family has been discovered only recently and further study is needed to focus on other possible pathways regulated by specific TLRs, such as the connection of TLR2 to the development of ALI and ARDS.

In summary, the association of toll-like transmembrane receptors with a large number of diseases creates a possibility for treatment with specific substances, compounds and biological particles. Although such a treatment could be beneficial for the patients, it seems to have a double nature, it could either trigger a strong autoimmune response resulting in diabetes, asthma and allergies or inhibit positive immune response against cancer cells or sepsis. Finding balance in regulation of TLRs pathway is crucial for efficient treatment.

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ABBREVIATIONS

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
COPD	Chronic Obstructive Pulmonary Disease
DC	Blood Dendritic Cells
dsRNA	double stranded RNA
FDA	Food and Drug Administration
HSP	Heat Shock Protein
IKKs	Iκβkinase kinase
IL1	Interleukin 1
IL6	Interleukin 6
IL8	Interleukin 8
IL12	Interleukin 12
INF-α	Interferon-α
IRI	Ischemia Reperfusion Injury
IRAK-4	IL-1 receptor–associated kinase-4
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat modules
MAL	T-lymphocyte maturation-associated protein
MyD88	Myeloid Differentiation primary response gene 88
NF-κB	Nuclear factor κB
PAMP	Pathogen Associated Molecular Pattern
PI3K	Phosphatidylinositol-3 Kinase
RA	Rheumatoid Arthritis
ssRNA	single stranded RNA
TAK1	TGF-β-activated kinase 1

TAB1	TAK1-binding protein 1
TIR	Toll Interleukin-1 Receptor
TLR	Toll-Like Receptor
TIRAP	TIR-domain-containing Adaptor Protein
TNF-α	Tumor Necrosis Factor α
Tollip	Toll Interacting Protein
TRAF6	TNF - associated factor 6
TRIF	TIR-domain-containing Adaptor Protein Inducing Interferon-β
TRAM	TRIF-related Adaptor Molecule
VEGF	Vascular Endothelial Growth Factor

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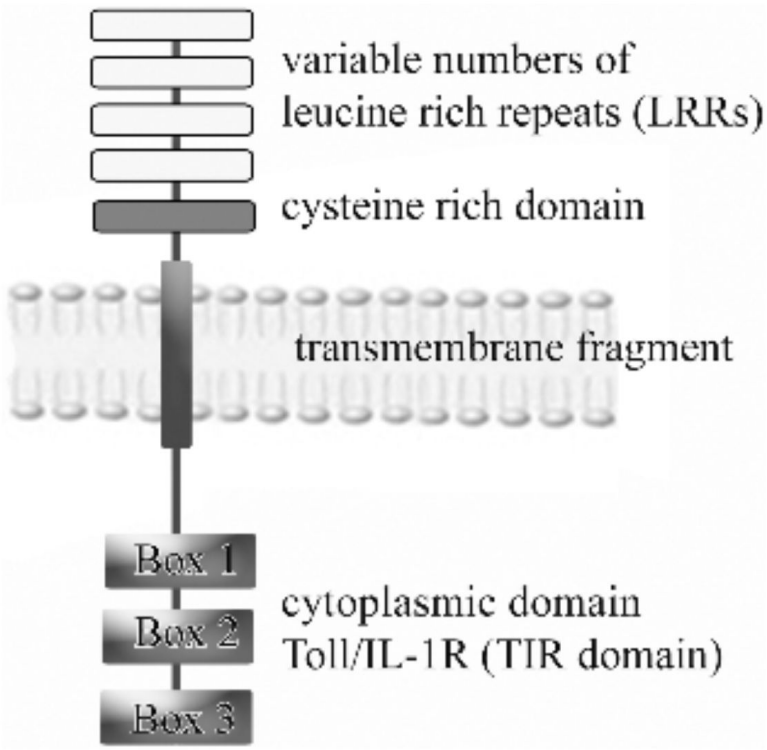

Fig. (1).

Structure of TLRs. One of major TLRs components is conserved cytoplasmic domain - Toll/IL-1R (TIR) domain, which contains three highly homologous regions (Box 1, Box 2 and Box3). This domain is linked by transmembrane fragment to variable number of N-terminal leucine rich repeats (LRRs) followed by a cysteine rich domain.

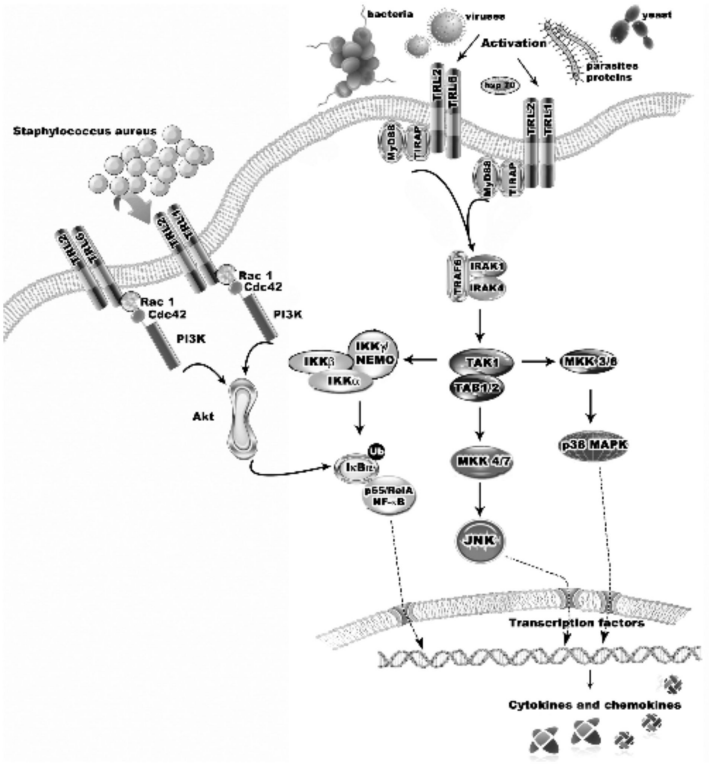

Fig. (2).

TLRs signaling pathway showed on TLR2 example. To be activated, TRL2 requires dimerization with TLR1 or TLR6. The signaling cascades *via* TIR domains are mediated by specific adaptor molecules including MyD88, TIRAP, TRIF, and TRAM. Upon ligand binding to TLRs, the adaptor molecule MyD88 is recruited to the TLR complex as a dimer. Binding of MyD88 promotes association with IL- 1, IRAK-4, and IRAK-1. TRAF6 is recruited to IRAK-1 and the IRAK-4/IRAK-1/TRAF6 complex dissociates from the receptor. It interacts with another complex consisting TAK1, TAB1, and TAB2. TAK1 is subsequently activated in the cytoplasm, leading to the activation of IKKs. IKK activation leads to the phosphorylation and degradation of IκB and consequent release of NF-κB. Translocation of NF-κB into the nucleus activates inflammatory chemokines and cytokines. The MyD88-independent pathway induces NF- B and MAPKs with delayed kinetics. It is possible that product of this pathway will have different targets from those activated by MyD88 dependent pathway. For example it can induce dendritic cell maturation instead cytokine production [136]. This pathway induces a fast and transient activation of the Rho GTPases - Rac1 and Cdc42. Recruitment of active Rac1 and phosphatidylinositol-3 kinase (PI3K) to the TLR2 cytosolic domain allows activation of Akt kinase, activation and translocation of the p65 subunit of NF-κB into the cell nucleus and specific cytokines release.

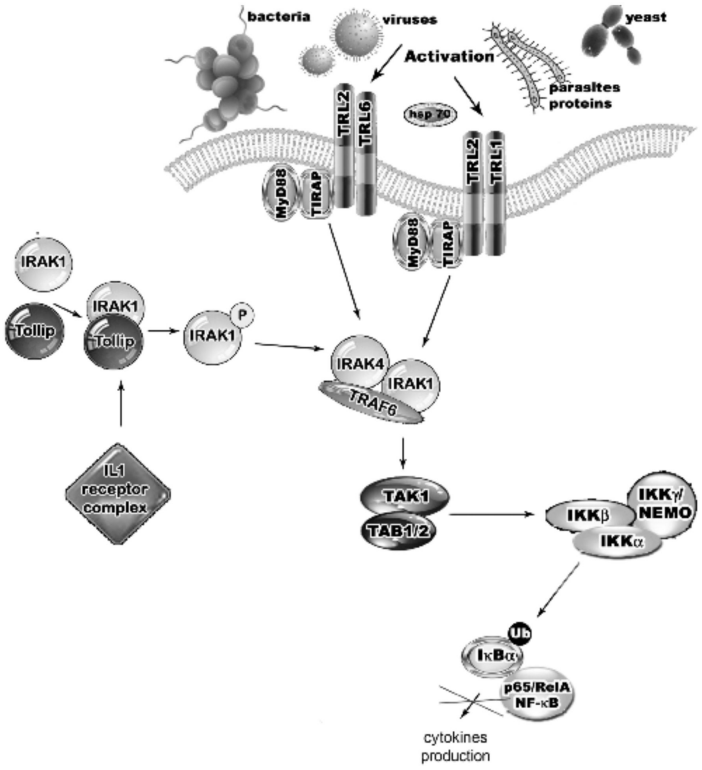

Fig. (3).

The TRL2 pathway is activated by Tollip and results in IRAK suppression. Upon stimulation with IL-1, the Tollip-IRAK-1 complex is recruited to the IL-1 receptor complex causing IRAK-1 phosphorylation, dissociation from Tollip and TRAF6 activation. Tollip overexpression leads to the suppression of NF-kβ inhibition. Negative regulation of TLR signaling by Tollip may therefore serve to limit the production of pro-inflammatory mediators during the inflammation process following an infection.

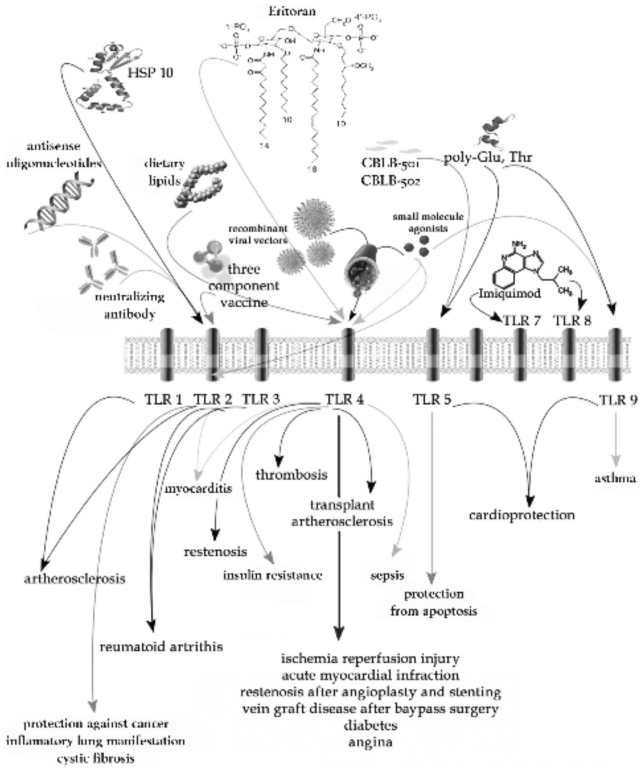

Fig. (4).

Scheme depicting drugs acting on designated TLRs with possible therapeutic effect and clinical significance in given disease.

Table 1

Members of TLR Family with Assigned Gene Location

Subfamily	Members	Gene Location
TLR2	TLR1	4p14
	TLR2	4q32
	TLR6	4p14
	TLR10	-------
TLR3	TLR3	4q35
TLR4	TLR4	9q33–35
TLR5	TLR5	1q33.3
TLR9	TLR7	Xp22
	TLR8	Xp22
	TLR9	3p21.3

Table 2

Characteristic of TLRs Showing Their Tissue and Cell Localization, Specific Ligand Interactions and Disease Involvement

Toll Like Receptor Type	Cell Type	Ligands	Involvement in the Disease Development
TLR1	• Neutrophils, eosinophils, • Mast cells • monocytes/macrophages • human blood dendritic cells • B cells • Keranocytes • Mucosal epithelial cells	• Soluble factors of *bacterial and mycobacterial* cell wall • G+, G− bacterial triacylated lipopeptides	
TLR2	• neutrophils, eosinophils, basophils • Mast cells • small airway epithelial cells • airway smooth muscle cells, • type II alveolar epithelial cells • tracheal muscle layer, • monocytes/macrophages • glial cells • murine bone-marrow derived mast cells • B cells • Human blood dendritic cells • NK cells • Keranocytes • Mucosal epithelial cells • Human endothelial cells	• G+ bacterial peptidoglycan, lipoteichoic acids • Phenol-soluble modulin • Di- and triacylated lipopeptides, lipoproteins • Outer-membrane porins • Outer-surface protein – OspA • factors of *mycobacterial* cell wall • Zymosan • Protozoan cell membrane glycolipids • Wild-type H protein • HSV-1, CMV viruses envelope proteins • Host HSP70 • Parasites proteins	• Inflammatory response in the pathogenesis of atherosclerotic plaque destabilization • Intimal hyperplasia after arterial injury • Innate immunity • Autoimmune diabetes mellitus (DM1A) • cardiomyopathy
TLR3	• Mature human blood dendritic cells • NK cells • Keranocytes • Mucosal epithelial cells	• Viral and host double-stranded RNA • Polyinosinic-polycytidylic acid	• Antiviral and immunostimulatory defense mechanism • Autoimmune diabetes mellitus (DM1A) • cardiomyopathy
TLR4	• Neutrophils, eosinophils, basophils • monocytes/macrophages	• G− bacterial lipopolysaccharide (LPS) • Fusion protein of respiratory syncytial virus	• Inflammatory response in the pathogenesis of atherosclerotic plaque destabilization • Intimal hyperplasia after arterial injury

Toll Like Receptor Type	Cell Type	Ligands	Involvement in the Disease Development
	• human blood dendritic cells • B cells • Keranocytes • Pulmonary epithelial cells • intestinal epithelial cells • corneal epithelial cells • Human endothelial cells	• Murine mammary tumour virus • Moloney murine leukaemia virus • Paclitaxel • Host extravascular fibrinogen/fibrin • Host oligosaccharide fragments of hyaluronan • Host extra domain A of fibronectin • Host polysaccharide fragments of heparan sulphate • Heat-shock protein 60 • Heat-shock protein 70	• unstable angina • asthma • Innate immunity • Systemic Lupus Erythematosus (SLE) • Autoimmune diabetes mellitus (DM1A) • cardiomyopathy
TLR5	• neutrophils • monocytes/macrophages • human blood dendritic cells • NK cells • Keranocytes • Basolateral intestinal epithelial cells • Mucosal epithelial cells	• Flagellin • Single stranded DNA	
TLR6	• Neutrophils, eosinophils, • Mast cells • monocytes/macrophages • B cells • Mucosal epithelial cells	• Diacylated lipopeptides • Zymosan	
TLR7	• Neutrophils, eosinophils, • monocytes/macrophages • myeloid human blood dendritic cells • B cells	• Imidazoquinolines • Flagellin • Single stranded DNA	
TLR8	• Neutrophils • monocytes/macrophages	• Viral single-stranded RNA • Guanosine and uridine-rich ssRNA HIV-1 oligonucleotides	
TLR9	• Neutrophils, eosinophils, • monocytes/macrophages • myeloid human blood dendritic cells • B cells	• Unmethylated CpG oligodeoxynucleotides • Viral genomic DNA	• Autoimmune diabetes mellitus (DM1A) • cardiomyopathy
TLR10	• Neutrophils, eosinophils, • monocytes/macrophages • B cells	• ***not yet identified***	

Toll Like Receptor Type	Cell Type	Ligands	Involvement in the Disease Development
TLR11	• Neutrophils • monocytes/macrophages • liver, kidneys, and bladder epithelial cells	• Uropathogenic bacteria • soluble extract of the tachyzoite	• preventing infection of internal organs of the urogenital system
TLR12	• Neutrophils • monocytes/macrophages	• ***not yet identified***	
TLR13	• Neutrophils • monocytes/macrophages	• ***not yet identified***	