Chapter

# Toll-Like Receptors, Keys of the Innate Immune System

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## Abstract

Toll-like receptors (TLRs) are members of the integral glycoproteins family, which are consist of intracellular and endoplasmic domains. TLRs are widely distributed in body tissues and expressed by immune and nonimmune cells. They are able to identify pathogens that cause cell injury and distinguish them from harmless microbes, and pathogenic nucleic acids as their binding ligand. Upon binding to their ligands, TLRs first underwent conformational changes; either forming homodimers or heterodimers, starting signaling pathways involve adaptor molecules utilization and then signal transduction through either myeloid differential (MyD)-88 dependent or independent pathways. Ending with activation of several transcription factors (TF) and release of pro-inflammatory cytokines (CK) and Type I interferons (IFN) and initiation of inflammation. TLRs are involved in almost all-inflammatory processes due to underlying disorders and diseases, which made them interesting targets for therapeutic development, via the synthesis of different agonists, antagonists, and even naturalized antibodies.

**Keywords:** Innate immune response, Toll-like receptors, Myeloid differential88, Cluster differential 14, Lymphocyte antigen 96 & Pro-inflammatory cytokines

## 1. Introduction

Our start point is that: inflammation is known pathogenesis of different pathophysiological conditions and diseases affecting different body tissues whether acute or chronic. Every inflammation involves an immune response -innate and adaptive- that started with specific receptors called recognition receptors to identify stimuli/damage signal, activation of consequence inflammatory pathway/cascade, the release of inflammatory markers, and recruitment of inflammatory immune cells [1].

The innate immune response is initiated by either endogenous ligands acting as damage signals known as the damage-associated molecular pattern (DAMPs), or exogenous pathogenic ligands-that are accurately portion of the pathogenic microorganism- lead to the same fate; damage signals throughout pathogen-associated molecular patterns (PAMPs) [2]. These patterns alter the body of the cell and cause tissue injuries leading to massive necrosis that release intracellular component into surrounding, these components activate TLRs [3, 4]. These processes, which are both the mechanism and the net results of inflammations, infections, or ischemic injuries cause more, harm than the initial causes itself by improper stimulation of the immune response [3, 4].

TLRs are a family of pattern recognition receptors (PPR), which also involves nucleotide oligomerization domain (NOD)-like receptors (NLR) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLR). They are located on cell membrane/surface and nucleus, are responsible for the detection/recognition of the pathogen or intracellular damaged derived molecular signals to start immune response [1, 2].

These complicated inflammatory processes induced by the immune system are the "Classical typical scenario" involved in the majority of ischemic events, cancers, infectious and inflammatory diseases [4]. For further information about the immune system, Video 1 (https://youtu.be/8mEnyBdsrr8) can be shown on Armando Hasudungan YouTube channel [2] that would explain the innate immunity link with TLRs.

## 2. Toll-like receptors

TLRs are PRR family involves 13 members that exist in mammals with 10 members detected in the human genome [5, 6], depending on their similar morphology with Toll. Toll is a gene product that participate in both embryonic polarity development and adult fly -antimicrobial response of the species *Drosophila melanogaster* [6, 7]. A 1996 study of this gene product linked the loss/gain of function to the insect's susceptibility and immunologic response to fungal infections; increasing the temptation to seek for the amino acids sequence of the genome. This lead to the final identification of toll-like receptors in 1998 [4].

### 2.1 Toll-like receptors tissue expression and cellular distribution

TLRs are expressed in almost all body tissues involved in immunologic response as well as those exposed to external environments like the spleen, blood, lung & gastrointestinal tract [4, 8]. The particular cellular expression involves innate and adaptive immunity as well as different nonimmune cells. TLRs cellular expression involves the white blood cells "the sentinel of the innate immune response": microphages (MΦ) & mast cell (MC) "innate immune response keys", dendritic cells (DCs) (primarily pathogenic detector of the adaptive immune response) [4, 6, 8, 9], endothelial cells, epithelial cells, fibroblast, glial cells, astrocytes, oligodendrocytes, etc. [1, 5, 8, 10].

Cellular expression of TLRs family members largely variable and mainly depends on the presence of active infections [8]; according to the same source, as ex., bacterial product & pro-inflammatory cytokines can induce the expression of TL3 while IL-10 blocks TLR4 expression. It has been found that TLR2 expression is more specifically involved in the gram-positive bacteria signaling [8]. TLRs are located either primarily to immune cell plasma membrane phospholipids including TLR 1, 2, 4, 5, 6, & 11 [3, 4, 8]; Or located at the endosomal and lysosomal phospholipids where their extracellular domain (ECD) and its ligand-binding site project into the interior of the organelles like TLR 3, 7, 8, 9, 10 and 13 [2, 3, 10, 11].

### 2.2 Toll-like receptors biochemistry and Structure

TLRs are a type I integral transmembrane glycoprotein family of very conserved structure [5, 7], consist of 700–1100 amino acids [2, 4]. Their structure, shown in **Figure 1** consist of 2 domains: an ECD that recognize ligands, consist of repetitive motifs rich with leucine and an intracellular domain (ICD) –called cytoplasmic- that maintain inflammatory signal consequence, the last consist of interleukin (IL)-1 receptor region called Toll/IL receptor (TIR) domain [12, 13].

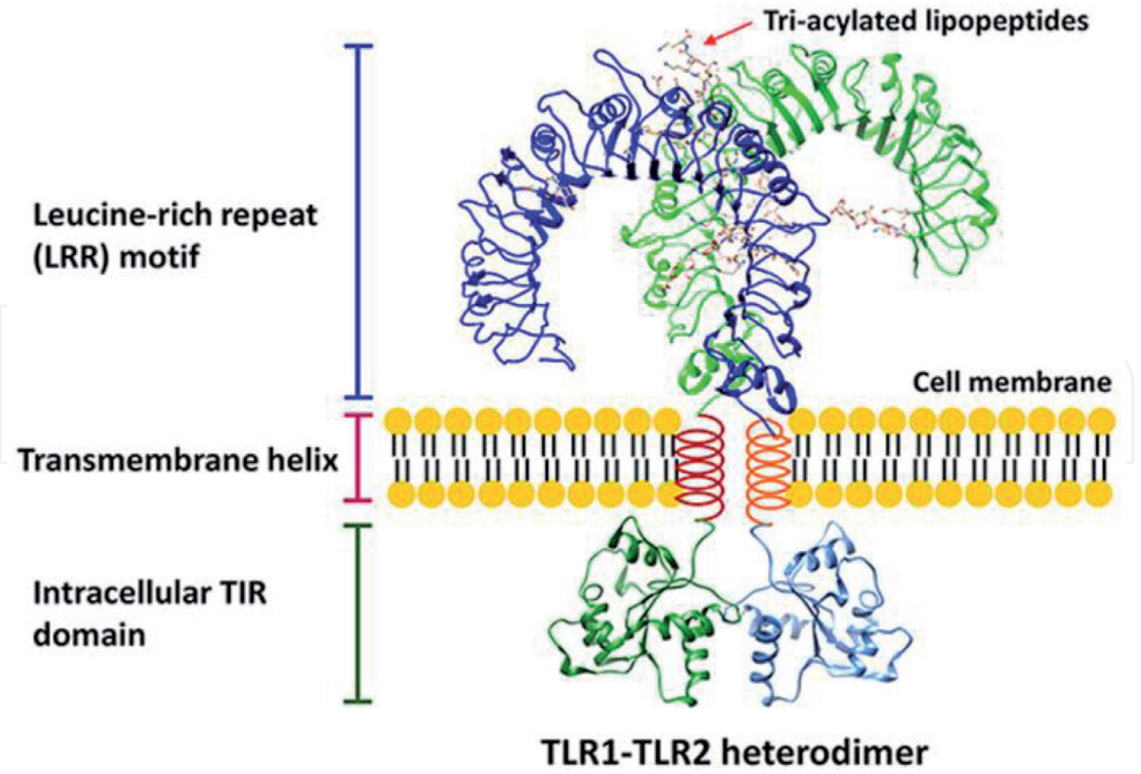


**Figure 1.**
*A representative structure of TLR. The conserved structural features of all TLRs consist of three critical components: (1) leucine-rich repeat (LRR) motif; (2) transmembrane helix; (3) intracellular TIR domain. The LRR structure is based on the model of TLR1-TLR2 heterodimer (Protein Data Bank, PDB, ID: 2z7x) interacting with six triacylated-lipopeptides, Pam3CysSerLys4 (Pam3CSK4), whereas the TIR domain homology model is based on TLR2-TIR structure (PDB ID: 1fyw) [12].*

## 3. Toll-like receptors family members

TLRs involves 13 family members that exist in mammals with 10 members detected in the human genome [5, 6]. Human TLRs amino acids sequence allow a subfamily classification into the TLR2, TLR3, TLR4, TLR5, and TLR9 subfamilies. The TLR2 subfamily involves TLR1, 2, 6, and 10; the TLR9 subfamily involves TLR7, 8, and 9 [14].

TLRs members can form homodimers/heterodimers among their same protein family or associates with an "outside TLR family" protein; both formations contribute to their structural and functional diversity [4]. Homodimers are formed by TLR4 while TLR members 1, 2, and 6 like TLR1/2 or TLR2/6 dimers form heterodimers [2, 3, 15–17]. TLRs members, their dimerization, cellular distribution, ligands, induced signaling pathway, and product are shown in **Table 1**; for further information about TLRs, Video 2 (https://youtu.be/8mEnyBdsrr8) about TLR overview can be shown at Armando Hasudungan YouTube channel [18].

### 3.1 Toll-like receptors binding ligands

TLRs family members can recognize two types of associated molecular patterns as their ligands, derived from pathogens or damaged organelles damaged structures.

#### *3.1.1 PAMPs*

PAMPs derived from pathogen [5, 19]; like gram-negative bacterial lipopolysaccharides (LPS), gram-positive bacterial lipoteichoic acid (LTA) and peptidoglycan

| TLRs | Immune Cell Expression | PAMPs | DAMPs | Signal Adaptor | Production |
|---|---|---|---|---|---|
| TLR1+<br>TLR2 | Cell surface<br>Mo, MΦ, DC, B | Tri-acylated lipoproteins (Pam3CSK4)<br>Peptidoglycans, Lipopolysaccharides | (TLR2 DAMPs listed below) | TIRAP, MyD88,<br>Mal | IC |
| TLR2+<br>TLR6 | Cell surface<br>Mo, MΦ, MC, B | Diacylated lipoproteins<br>(FSL-1) | Heat Shock Proteins<br>(HSP 60, 70, Gp96)<br>High mobility group proteins (HMGB1)<br>Proteoglycans<br>(Versican, Hyaluronic Acid fragments) | TIRAP, MyD88,<br>Mal | IC |
| TLR3 | Endosomes<br>B, T, NK, DC | dsRNA (poly (I:C))<br>tRNA, siRNA | mRNA<br>tRNA | TRIF | IC, type1 IFN |
| TLR4 | Cell surface/<br>endosomes<br>Mo, MΦ, DC, MC,<br>IE | Lipopolysaccharides (LPS)<br>Paclitaxel | Heat Shock Proteins<br>(HSP22, 60, 70,72, Gp96)<br>High mobility group proteins (HMGB1)<br>Proteoglycans<br>(Versican, Heparin sulfate,<br>Hyaluronic Acid fragments)<br>Fibronectin, Tenascin-C | TRAM, TRIF<br>TIRAP, MyD88<br>Mal | IC, type1 IFN |
| TLR5 | Cell surface<br>Mo, MΦ, DC, IE | Flagellin | | MyD88 | IC |
| TLR7 | Endosomes<br>Mo, MΦ, DC. B | ssRNA<br>Imidazoquinolin-es (R848)<br>Guanosine analogues (Loxoribine) | ssRNA | MyD88 | IC, type1 IFN |
| TLR8 | Endosomes<br>Mo, MΦ, DC, MC | ssRNA,<br>Imidazoquinolines (R848) | ssRNA | MyD88 | IC, type1 IFN |
| TLR9 | Endosomes<br>Mo, MΦ, DC, B, T | CpG DNA<br>CpG ODNs | Chromatin IgG complex | MyD88 | IC, type1 IFN |
| TLR10 | Endosomes<br>Mo, MΦ, DC | profilin-like proteins | | MyD88 | IC |

*Mo: monocytes, MΦ: macrophages, DC: dendritic cells, MC: Mast cells, B: B cells, T: T cells, IE: Intestinal epithelium, IC: Inflammatory cytokines [2]*

**Table 1.**
*TLRs cellular expression, binding ligands, signal adaptor & production [2].*

(PGN), mycobacterial lipopeptides, yeast zymosan, viral and bacterial ribonucleic acid (RNA), and unmethylated cytosine phosphate guanine containing- (CpG) deoxyribonucleic acid (DNA) [20, 21].

*3.1.2 DAMPs*

DAMPs damaged organelles structures, extracellular matrix, cytosolic and nuclear proteins, Heat shock protein-60 (HSP-60) and HSP-70, hyaluronic acid fragments, and free fatty acids (FFA) [5, 22, 23]. They cause activation of the innate and inflammatory immune responses, epithelial regeneration, and sterile inflammation control [6, 24].

## 4. Toll-like receptors signaling pathway

Upon TLRs recognition and binding to their ligands, they undergo conformational changes, dimerization as well as interaction with adaptor molecules passing series of intracellular signal transduction pathways that involve transcription factors NF-κB, IRFs, and mitogen-activated protein kinase (MAPK) activation. These pathways finally resulting in the secretion of pro-inflammatory mediators including nitric oxide (NO), CK- like tumour necrosis factor-alpha (TNF-α), IL-6 & IL-1β, chemokines (CC), and type I IFN [15, 21, 25, 26]. As shown in **Figure 2**.

### 4.1 Co-receptors

Co –receptors involved in TLRs signalling include Cluster differential 14 (CD14) and Lymphocyte antigen 96 (MD-2). Both have a major role in TLR4 activation after LPS recognition. CD14 is a glycophosphatidylinositol attached

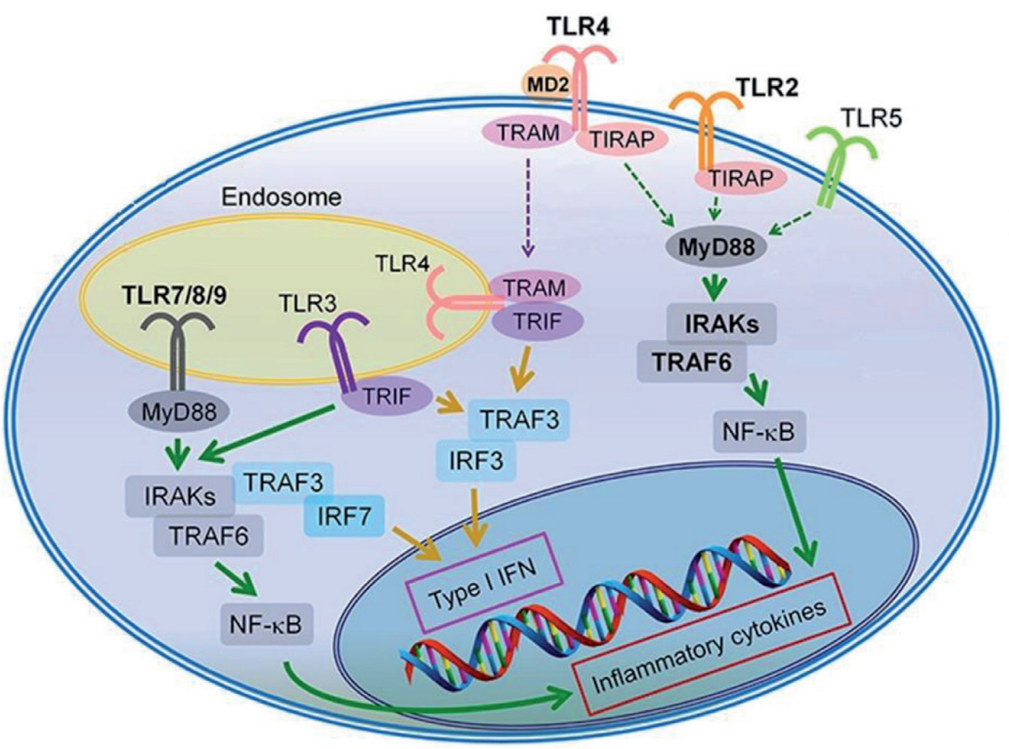


**Figure 2.**
*Signaling pathways of TLR. Surface and endosomal TLRs bind to adaptor molecules and co-receptors. Signal through Myd88 dependent/independent pathway ending with proinflammatory CK or type I IFN [12].*

protein expressed on innate immune cells as macrophage and monocytes that function as co-receptor for both cell surface & endosomal expressed TLRs. Lymphocyte antigen 96 (MD-2), which is a cell membrane glycoprotein associated specifically with TLR4 ECD, and expressed at myeloid and endothelial cells [6, 13, 21, 26, 27].

### 4.2 Adaptor proteins and kinase molecules

TLRs signaling pathways involves four main adaptor protein molecules: MyD88, TIR domain-containing adaptor protein/MyD88 adaptor-like molecules (TIRAP) also called MAL, TIR domain-containing adaptor protein inducing interferon-β (TRIF), and TRIF related adaptor molecule (TRAM) [13, 21, 28]. TLRs signaling pathways involves activation of five TIR containing adaptor kinase molecules, like IL-1 receptor-associated kinase (IRAK) -1 and 4, TNF receptor-associated factor-6 (TRAF6), serine/threonine binding kinase (TBK)-1, MAPK, and inhibitor of kappa-B (IκB) kinase (IKK) [13, 28].

### 4.3 Transcription factors

There are three transcription factors involved in the TLRs signalling pathway including NF-κB, AP1, and IRF. NF-κB is an intracellular pleiotropic protein complex; it is responsible for gene regulation of proinflammatory CK, CC, adhesion molecules, and cell cycle/survival regulating proteins as cyclin D1 and B cell lymphoma 2 (Bcl-2). AP1 is a dimer of both protein Jun and Fos families; that is associated with cell replication and survival regulation. Finally, the IRFs protein regulating IFNs, are responsible for signal stimulation via MyD88independent/TRIF pathway [6, 13].

### 4.4 Intracellular signaling pathways

There are two intracellular signalling pathways for TLRs involve MyD88-dependent/& MyD88-independent also called (TRIF-dependant) signal transduction pathway.

#### *4.4.1 MyD88-dependent pathway*

It is utilized by all TLRs but not TLR3 [21, 29]. This pathway activates the IRAKs, TRAF6, transforming growth factor (TGF)-β-activated kinase (TAK)-1 and the IKK complex [15]. It causes the nuclear translocation of NF-κB and adaptor protein-1 (AP1) [28, 30], and ends with the secretion of CK like IL-6, IL-10, IL-12 & TNF-α [16, 29]. MyD88 also stimulate the classical extracellular signal-regulated kinases (MAPK/ERK), phosphoinositide-3 (PI3), and Jun (N)terminal kinase (JNK) which stimulate the AP1 signalling pathway, and induce the interferon regulatory factor-7 (IRF7) ending with the release of type-I IFN or co-stimulatory molecules associated with the antimicrobial response by endosomal TLRs 3, 7, 8 and 9 [13, 29, 31, 32].

#### *4.4.2 MyD88-independent pathway*

The main pathway of TLR3 and 4, involve TRIF signalling pathway activation which involves TRAF6 activation, results in inositol triphosphate-3 (IP3) phosphorylation and induction of IFN-β gene expression as well as activation of TRAF6 [21, 29].

Surprisingly the same outcome was obtained from plasmatoid dendritic cells (pDCs) stimulated by TLR 7& 9 throughout the activation of the MyD88/IRF7 dependent pathway [15, 33].

### 4.5 Unique pathways

TLR4 further utilizes TIRAP to activate MyD88 and TRAM to bridge the TRIF activation, which means that TLR4 uniquely utilizes both the MYD88 dependent and independent pathways [11, 21, 29].

As stated by S. Kiziltas et al. "TLR stimulation product is dependent on the nature of PAMPs, the activated TLR, the activated cell and the level of CK. Moreover, the chronically activated signalling pathway would possibly induce transcription of oncogenic factor; adding further complexation to the intracellular signalling for these receptors" [5, 13].

## 5. Toll-like receptors and pathophysiological disorders

TLRs play an important role in pathophysiological disorders due to their wide tissue distribution, their function as pattern recognition receptors that respond to variable bacterial and damage associated molecules, and involvement in multiple inflammatory signal pathways/& process all render TLRs being a major player in any inflammation-related disorder [4–6, 19, 22, 23, 34]. In addition, analysis of TLRs gene polymorphism in human disorders revealed an increased risk of bacterial infection and sepsis as an example [34]. This section is a shortcut or summary to TLR involvement in different pathophysiological disorders rather than a full description section.

### 5.1 Toll-like receptors and pathophysiology of inflammatory oxidative stress

Inflammation is a common etiology of many disorders and disease including ischemic injuries, microbial infections, diabetes, arthritis and cancer [3, 4, 35]; still, any inflammatory process is triggered by damage signal recognized by pattern receptors and induce activation of signaling pathways leading to the production of pro-inflammatory markers and activation of immune cells [35]. These processes also induce the release of free radicals (FR) such as reactive oxygen species (ROS) and the activation of hypoxia-inducible transcription factor-1 (HIF1), causing tissue stress and reduced tissue oxygen status, so-called tissue hypoxia. Hypoxia is believed to be a hallmark as well as a key trigger of inflammation itself [35, 36].

Under normal conditions HIF1-α subunit (the inducible form of the heterodimer protein HIF-1 transcription complex) [35], is controlled by hydroxylation of proline residue via prolyl hydroxylase enzyme, and breaking down via proteasome. However under inflammatory conditions LPS activate TLRs that stimulates nicotine amide adenine dinucleotide phosphate (NADPH) oxidase (Nox)-associated crosstalk with the MAPK signaling pathways [36, 37], that causes proinflammatory CK & markers production thus increasing mitochondrial FR release like ROS causing more and more tissue stress. That causes HIF1- α activation; here HIF1- α protein inactivation process will be inhibited due to proline consumption, leading to HIF1-α accumulation in MΦ, DCs and other non-immune cells that exposed to hypoxia/ & non-hypoxic damage signals [38]. Furthermore, this would induce metabolic reprogramming of mitochondrial respiration causing succinate release, and production of IL-1β [35, 38].

In dendritic cells, TLRs cause further stabilization of HIF1-α via release of NF-κB, which would further increase glucose uptake and render shifting of mitochondrial respiration to the anaerobic glycolytic pathway due to the increased oxygen demand versus the decreased supply [35, 36]. Finally result in disruption of the normal function of DCs, the primary pathogenic detector of the adaptive immune response; which undergo cellular maturation upon TLRS activation that results in further expression of co-stimulatory molecules, further production of pro-inflammatory CK & CC, and migration to lymph node so to present antigens to naïve T-cells [4, 35]. All these scenarios would further amplify the existing inflammation and tissue damage [35].

HIF1-α is a transcription factor that responsible for cellular adaptive responses after exposure to injury/stress environment, including maintenance like controlling angiogenesis to improve blood vessel formation, shifting cellular mitochondria respiration to anaerobic glycolysis through improving cellular survival and cellular adhesion in oxidative stress environment's [36]. In addition, it is the major controller of phagocytes bactericidal capacity, and involved in myeloid cell-mediated inflammation, and is an essential factor for inhibition of myeloid cell apoptosis induced by LPS. The last point made it an important factor also in the TLR4 signaling pathway [36, 38]. HIF1-α function as a double-edged sword, that mediate cellular adaptive to stress but progress disease status by the same time [38].

## 5.2 Toll-like receptors important role in the pathophysiological disorder

### *5.2.1 Central nervous system*

TLRs are expressed in various central nervous system (CNS) cells predominantly in neurons, astrocytes, resident microglia, cerebral microvasculature, plexuses choroid, and leptomeninges. They are associated with the detection of- and regulated by central DAMPs [33]. TLR4 is further upregulated centrally by glutamate via N-methyl-D-aspartate (NMDA) dependent mechanism and peripherally by noradrenaline/β2 receptor, & corticotrophin-releasing factor. TLRs play an important role in restoring central homeostasis, physiology of stress-sensitive behaviour after injuries or diseases as multiple sclerosis, Alzheimer's, and stroke [33].

In the experimental model of CNS, stress exposure revealed mRNA upregulation and activation of TLRs in the brain frontal cortex after the stress is involved in the loss of neuronal plasticity and survival depending on the activation of NF-κB induced ROS production. Also resultant bacterial translocation from the gut to the systemic circulation and other organs such as the liver, spleen, and mesenteric lymph nodes; These circulating gram-negative bacteria are the major source of LPS, which can activate brain TLR4 through multiple pathways, including a neuroinflammatory response. This is partially explained by the theory known as leaky gut [11, 33].

In another experimental model of neurogenesis, TLR3 & 4 were found to act as down regulators, TLR3 deletion/loss of function was also linked to improved cognitive function. The same reference state an opposed case in viral meningitis when TLR3 & 9 recruitment help to decrease neuronal injury and localize infection area and in Alzheimer disease where TLR2, 4, 5, 7 & 9 were suggested to improve disease progression by inhibiting amyloid plaque accumulation [1].

### *5.2.2 Respiratory system*

TLR is thought to play a considerable role in several respiratory disorders starting from allergic rhinitis ending with severe inflammatory disorders like acute respiratory distress syndrome (ARDS), through their activation by the causative

inflammations derived by pulmonary oedema, trauma, sepsis & even drug overdose [9, 37]. In allergic rhinitis TLR2, 3, & 4 were found to be both upregulated by- and involved in-the causative inflammation [37].

TLR2 has the mainstay of involvement & determination in respiratory allergic disease due to considerable genetic variation. In asthma, an experimental study shows TLR2 induction by synthetic Pam3Cys triggers immune response & disease severity [37]. While in acute lung injury (ALI) & ARDS, TLR2 was found to be activated by Toll interacting protein (Tollip) [14]. TLR4 was found to increase asthmatics severity & prevalence in paediatrics. TLR4 genetic polymorphism affects cluster differentials (CD)41–251 regulatory T cells (Tregs) which are activated by LPS, the same ligand of TLR4 itself. [2, 3, 37].

*5.2.3 Cardiovascular system*

An experimental model of doxorubicin and hydrogen peroxide-induced cardiac injury showed TLR2 to be involved in cardio myocytes apoptosis, besides TLR2 targeting suggested to be protective in septic cardiomyopathy [1]. In addition, murine models revealed cardiac tissue expression of TLR4 increased after hypertension, myocardial ischemia, maladaptive left ventricular hypertrophy, and angiotensin II (AngII) infusion participating in vascular remodelling & stiffness, endothelial dysfunction, increase myocardial infarction (MI) size & susceptibility. While Human studies revealed the same in patients with unstable angina, MI, heart failure, atherosclerosis & myocarditis [9, 27, 39–41].

TLR4 expression & signalling was increased in patients' monocytes during attacks of unstable angina & MI [37]. In the experimental model & human vascular inflammation, TLR4 was found to increase the production of CK, CC as well as increase TLR2 expression. In the early stage of the atherosclerotic lesion, TLR4 mRNA protein was detected & MyD88 -the mainstay of TLR signalling pathway-gene deficiency was linked to decrement in CK, CC & lipid content production, as well as in atherosclerotic lesion size. The same reference stated that TLR2 genetic polymorphism was linked to increased coronary artery stenosis, while TLR7 & 8 was involved in cardiac inflammation caused by the Coxsackie virus [37].

*5.2.4 Gastrointestinal system*

The liver is the major organ that deals with gut-derived endotoxin, exposed by portal circulation [13, 42]. This continuous exposure would trigger frequent activation of the hepatic innate immune system; which contributes to the induction of inflammation in acute hepatic injuries, which means involvement of TLRs in the induction of inflammation [13]. Pathogenic suppression/& inhibition of TLRs found to mediates chronic hepatic injuries/disorders like hepatitis, fibrosis, alcoholic liver injuries, ischemia/reperfusion injury, and carcinoma [13, 28].

In Paracetamol human hepatotoxicity, endogenous chemical injury derives extracellular matrix (ECM) the ligand that activates TLR4 to release TNF-α, induce inducible nitric oxide synthase (iNOS), peroxynitrite, glutathione depletion, so that will amplify immune response, sequestering leukocytes, increase serum hyaluronic acid, causing steatosis, necrosis, and hepatic congestion [16].

Hepatitis viral nucleic acid & proteins are the ligands detected by TLR3, 7, 8, & 9. Starting with hepatitis B virus (HBV), in vitro activation of TLR1, 2, 3, 4, 5, 6, 7, 8, & 9 result in the release of IFN which inhibit HBV DNA replication and RNA transcription. Whilst HBV itself downregulates the expression of TLR1, 2, 4, & 6, this limits their antiviral effect or even renders them nugatory [28]. This downregulation of TLRs is attributed to the presence of HBV e antigen (HBeAg)

during acute infection. About hepatitis C (HCV), its core protein activates TLR 1, 2, 4 & 6, which are supposed to produce antiviral IFNs as well as increased hepatic inflammation. The same effect is presumed by TLR 3 & 4 in HBV is achieved here to produce IFN-β [28].

In alcoholic liver disease (ALD), alcohol mainstay effects are to increase gut mucosal permeability to LPS, modification of gut flora, reducing endotoxin clearance rate, and increasing hepatic endotoxin level [16]. These scenarios lead to higher expression of TLR1, 2, 4, 6 & 9 by both parenchymal and non-parenchymal cells, activating their pathway and release of inflammatory mediators, this process observed in the chronic alcohol experimental model [28, 29]. While a patient with cirrhosis expresses a high level of TNF-α, IL-1β, & IL-6, as well as chronic endotoxemia, recurrent bacterial infection [16]. Finally, the process of hepatic regeneration depends on the interplay between the immune system and non-parenchymal cell, which involves activation of TLRs/MyD88 pathway, here the bulky activation of TLRs, would inversely affect the regeneration process, which indicates that the extent of such activation is essential for hepatic regeneration. TLR2, 4 & 9 reported no important role in liver regeneration process [28, 43].

Both human patients and experimental models of diabetes linked the active TLR to the progression of diabetes complication throughout the activation of NF-κB signalling in adipose tissue MΦ due to high level of plasma FFA associated with obesity & diabetes type 2 (T2DM) [44].

In vivo & in-vitro studies performed by Zhang N. et al. revealed that TLR 2 & 4 activation in insulin target tissues as the liver, adipose tissue & immune cells linked them with insulin resistance. The first suggests that high TLRs loss of function or genetic modification protects against high FFA level resulted from large mass adipose tissue secreting non-esterified free fatty acids & reduction of their clearance/oxidation which disturbs gut permeability to LPs [45].

TLR4 resultant inflammation associated with activation IKK, MAPK, JNK, and p38 pathways would further increase insulin receptor substrate-1 (IRS1) serine phosphorylation thus decrease insulin receptor's signal transduction [31, 45]. Furthermore, TLR4-MyD88 signalling pathway activation was suggested throughout developmental researches for several anti-hyperlipidemic medications, while TLR1, 2, 3 & 7 were triggering both host immune defence and/autoimmune response that aggravate diabetic state [37].

*5.2.5 Urinary system*

TLRs expression in renal tube epithelial lining render their activation to be essential in renal vascular remodelling, endothelial dysfunction in multiple renal disorders like acute kidney injury (AKI), solid organ transplant, glomerulonephritis, ischemic/reperfusion injury (I/R injury) & diabetic renal disorders [27, 44]. Experimental streptozocin induced diabetic model revealed podcytopathy & fibrosis regression after TLR4 knocking out, as they are expressed by podocytes & decreased diabetic nephropathy after TLR2 knocking out [46, 47]. TLR4 gene polymorphism was linked to prostate cancer among gene clusters of TLR1, 6 & 10 [37].

## 6. Toll-like receptors as therapeutic targets

TLRs, as the primary receptor for many ligands that trigger innate & adaptive immune response, with complex signaling pathways involving many adaptor

molecules & co-receptors seem interesting for therapeutic target development. Synthetic agonist, antagonist and even naturalized antibodies could modify TLRs signaling to make them attractive targets for the management of different inflammatory disease. For example at 2013, Savva and Roger enlisted around 32 clinical trials at different phases for TLRs agonist/antagonist agent for the management of sepsis and infectious disease, these trials include even the antimalarial old agent chloroquine [28, 34].

### 6.1 Toll-like receptors 1 and 2

TLR1/2 heterodimers were found to be increased in patients with atherosclerotic lesions, while administration of TLR1/2 agonist aggravates disease status, also TLR2 inhibition was suggested as diabetes and cardiovascular disorders therapy besides statins & thiazolidinedione by anti-inflammatory action [9]. Pam2/3CSK4 TLR2 ligands covalently linked to CD8+ or B-cell epitopes associated peptides were found to enhance therapeutic response in tumour models, by stimulating TLR2 induced T-cell activation [15]. A 3 component carbohydrate-based cancer vaccine involved TLR2 activator that mediates humoral immune response against tumour-induced glycopeptide antigens by affecting the maturation of cellular component of the innate immune system (DC & natural killer cells), furthermore cancer treatment with chimers of anti-tumour antibodies and small molecule agonist of TLR2 would alleviate disease progression [9].

### 6.2 Toll-like receptor 3

Since high synovial expression of TLR3 in RA patients was found, one scenario for rheumatoid arthritis and possibly bone malignancy is to inhabit the TLR3 pathway via the RNA synthetic analogue Polyinosine-polycytidylic acid (poly (I:C) that affect monocyte –osteoclast cellular differentiation [9].

### 6.3 Toll like receptor 4

Various TLR4 antagonist was developed as a therapeutic agent, starting with the peptide P13- an inhibitor of TIR domain signalling pathway- that was found to ameliorate inflammatory response and improve surviving in a TLR4-mediated hepatic injury of murine model [16]. In addition, Lipid A mimetics E5564 and CRX526 bind to TLR4-MD2 complex showing valuable inhibition of pro-inflammatory cytokine IL-1 and TNF-α production in LPS treated animal models as well as septic shock patients in phase III clinical trial [9, 16, 29]. TLR4 inhibition was suggested as the scenario for treatment of thrombosis, atherosclerosis & vascular restenosis throughout coating TLR4 or MyD88 with inhibitory compound, small molecule antagonist, then by giving viral vectors that express antisense gene to TLR4 RNA [9], and finally TLR4/MD2/anti-Human IgG (Fc specific) (IgG-Fc) fusion protein inhibitor of NF-κB and JNK activation provides interesting biologic therapy for liver fibrosis, alcoholic and non-alcoholic steatohepatitis by decreasing IL-6 and monocyte Chemoattractant Protein-1(MCP-1) production [16].

Another TLR4-synergizer Fc/fusion protein and TL4 ligand α-1 acid glycoprotein were found to inhibit LPS-induced activation of hepatic MΦ by blocking the triggering receptor expressed on myeloid cells-1 (TREM1), and boosting the anti-inflammatory immune response. Other theoretically interesting scenarios involving the I.V administration of monophosphoryl lipid A derivatives as 2 adult HBV vaccine in treating viral hepatitis [13, 15, 16].

### 6.4 Toll-like receptors 5 and 7

One possible scenario for cancer immunotherapy involved TLR5 binding to flagellin that can turn the tolerogenic DCs into active antigen-presenting cells (APC) [9].

Isatoribine, a TLR7 agonist administered I.V was found to decrease viral load with a moderate adverse effect profile in HCV patients. In addition, IGS-9620 that was experimentally assessed on the HBV animal model was found to decrease HBVs antigen (HBsAg) level in serum, HBV viral load as well as IFN-α in dose dependent-manner [15, 29]. Note that some TLR7 targeting therapies were approved by Food and Drug Administration (FDA) like imiquimod, TLR7-immune response modifier that was approved since 1997 for treatment of superficial skin malignant melanoma & genital warts by increasing cellular production of CK like IFN, IL-6 & TNF [9].

### 6.5 Toll-like receptor 9

Selective TLR9 agonists like 1018 ISS (immunomodulatory sequences) that contain repeated CpG motifs were found to modulate the TLR9 signalling pathway involved in HBV infection and have been tested in phase III clinical trials. Another agonist IMO-2055 was under assessment in 2011 for oncologic disease as well as IMO-2125 which was found to maintain the high level of IFN was under assessment as a possible therapy to HCV patients. The TLR9 intracellular signalling inhibitors ST2825 and RO0884 designed to block IRAK1 &4/MyD88 singling pathway caused inhibition of the NF-κB, IL-1β, and TNF-α activation as well as decreased hepatic IL-6 secretion [9, 15, 29].

## 7. Conclusions

Medical and pharmacological development is focusing on the molecular level, in all aspects including analytical, physiological, pharmacological and even genetic aspects. Understanding immune response is thus important subject, furthermore, the target receptors which damage signals bind to, their signaling pathways end products will tell what possible immune response happened to human body. Toll-like receptors are those targets, the family of integral transmembrane glycoprotein expressed intracellularly or at cellular surface, considered main component and link between innate and adaptive immune response, which can induce signaling pathways involving four main adaptor molecules that initiate divaricated steps ending with inflammatory cytokines. These pathways could be involved in any inflammatory process/disorders and thus seems interesting targets for pharmacological intervention; all these steps bring us back to the bullet that explodes all these events in the body, the immune system.

## Conflict of interest

The author declares no conflict of interest.

## Notes/thanks/other declarations

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## Abbreviations and nomenclature

| | |
|---|---|
| AKI | Acute kidney injury |
| ALD | Alcoholic liver disease |
| ALI | Acute lung injury |
| AngII | Angiotensin II |
| AP-1 | Adaptor protein-1 |
| APC | Antigen-presenting cell |
| ARDS | Acute respiratory distress syndrome |
| Bcl-2 | B cell lymphoma 2 |
| CC | Chemokine |
| CD14 | Cluster differential 14 |
| CK | Pro-inflammatory cytokines |
| CpG | Cytosine phosphate guanine |
| DAMPs | Damage-associated molecular pattern |
| DC | Dendritic cell |
| ECD | Extracellular domain |
| ECM | Extracellular matrix |
| FFA | Free fatty acids |
| FR | Free radicals |
| HBeAg | HBV e antigen |
| HBsAg | HBV s antigen |
| HIF1 | Hypoxia-inducible factor-1 |
| HSP | Heat shock protein |
| I/R injury | Ischemic/reperfusion injury |
| ICD | Cytoplasmic domain |
| IFN | Type-I interferon |
| IgG-Fc | Anti-Human IgG (Fc specific) |
| IKK | Inhibitor of kappa-B (IκB) kinase |
| IL | Interleukin |
| iNOS | Inducible nitric oxide synthase |
| IP3 | Inositol triphosphate-3 |
| IRAK | IL-1 receptor-associated kinase |
| IRF | Interferon regulatory factor |
| IκB | Inhibitor of kappa-B |
| JNK | Jun (N)terminal kinase |
| LPS | Lipopolysaccharides |
| LRR | Leucine-rich repeat |
| LTA | Lipoteichoic acid |
| MAPK | Mitogen-activated protein kinase |
| MAPK/ERK | Extracellular signal-regulated kinases |
| MC | Mast cell |
| MCP-1 | Monocyte Chemoattractant Protein-1 |
| MD-2 | Lymphocyte antigen 96 |
| MI | Myocardial infarction |
| mRNA | Messenger ribonucleic acid |
| MyD88 | Myeloid differential88 |

| | |
|---|---|
| MΦ | Macrophage |
| NADPH | nicotine amide adenine dinucleotide phosphate |
| NLR | nucleotide oligomerization domain (NOD)-like receptors |
| NO | Nitric oxide |
| NOD | nucleotide oligomerization domain |
| Nox | NADPH oxidase |
| Pam3CSK4 | Pam3CysSerLys4 |
| PAMPs | Pathogen-associated molecular patterns |
| pDCs | Plasmatoid dendritic cells |
| PGN | Peptidoglycan |
| PI3 | Phosphoinositide-3 |
| poly(I:C) | Polyinosine-polycytidylic acid |
| PRRs | Pattern recognition receptors |
| RIG-I | retinoic acid-inducible gene I |
| RLR | retinoic acid-inducible gene I (RIG-I)-like receptors |
| ROS | Reactive oxygen species |
| T2DM | Diabetes type 2 |
| TAK | Transforming growth factor (TGF)-β-activated kinase |
| TBK-1 | Serine/threonine binding kinase |
| TF | Transcription factors |
| TGF | Transforming growth factor |
| TIR | Toll/IL-receptor |
| TIRAP/MAL | TIR domain-containing adaptor protein/MyD88 adaptor like |
| TLRs | Toll-like receptors |
| TNF-α | Tumour necrosis factor-alpha |
| Tollip | Toll interacting protein |
| TRAF6 | TNF receptor-associated factor-6 |
| TRAM | TRIF related adaptor molecule |
| Tregs | Regulatory T cells |
| TREM1 | Triggering receptor expressed on myeloid cells-1 |
| TRIF | TIR domain-containing adaptor protein inducing interferon-β |

## Video materials

### Vedio 1. Immunology-Toll Like Receptors Overview

YouTube video: [3] Armando Hasudungan, Immunology-Toll Like Receptors Overview [Internet. YouTube]. 2014. Available from: https://youtu.be/8mEnyBdsrr8

### Vedio 2. Toll Like Receptors Overview

YouTube video: [18] Armando Hasudungan, Immunology - Toll Like Receptors Overview [Internet YouTube]. 2014. Available from: https://youtu.be/8mEnyBdsrr8

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