

# Inflammasomes in inflammatory disorders: the role of TLRs and their interactions with NLRs

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**Abstract** The innate immune system relies on a variety of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) to sense microbial structures that are present in pathogens. Various levels of crosstalk between the TLR and NLR pathways have been described, most notably the description of a molecular scaffold complex, termed the inflammasome, which requires input from both pathways and leads to the activation of the proinflammatory cytokines interleukin (IL)-1 $\beta$  and IL-18. In certain cases, the inflammatory process becomes dysregulated and chronic inflammatory diseases may develop. Understanding the interactions of the TLR and NLR pathways will provide further clues to the pathogenesis of these diseases and to the development of efficient therapies to combat them.

**Keywords** TLR · NLR · PRRs · Innate immunity · Inflammatory disorders

## Introduction

A key function of the innate immune system is to recognize invading microbes and mount a quick and effective response. This is done through a variety of receptors, referred to as pattern recognition receptors (PRRs) that recognize molecular patterns on pathogens called pathogen-associated molecular patterns (PAMPs). This review examines two types of PRRs, namely, Toll-like receptors (TLRs) and NOD-like receptors (NLRs), and the interplay between them.

## Toll-like receptors

TLRs are arguably the most studied of the PRRs. They are transmembrane receptors that recognize individual PAMPs on invading microbes. Recognition of PAMPs by the TLRs triggers a series of events that leads to the expression of many immune and inflammatory genes. TLRs also induce dendritic cell maturation, which is essential for the induction of pathogen-specific adaptive immune responses. To date, 10 TLRs (TLR1-10) in human and 12 TLRs (TLR1-9 and TLR11-13) in mice have been described [1].

All TLRs are type 1 transmembrane glycoprotein receptors. The extracellular domain contains 19–25 consecutive leucine rich repeat (LRR) motifs, each of which is 24–29 amino acids in length, and are involved in ligand binding. The intracellular tail contains a highly conserved region, called the Toll-interleukin-1 receptor (TIR) domain, which mediates interactions between TLRs and downstream signaling molecules [2].

TLRs are expressed on a range of immune cells, which include macrophages, dendritic cells, B cells, and certain types of T cells. TLRs are also expressed on certain nonimmune cells, such as epithelial cells, which lie at potential sites of entry, including the skin, respiratory, intestinal and genitourinary tracts, endothelial cells and smooth muscle cells. Their pattern of expression is modulated by activation, maturation or differentiation of the different cell types. TLRs 3, 7, 8, and 9 are expressed intracellularly, while TLRs 1, 2, 4, 5, 6, and 10 are expressed on the cell surface.

TLRs recognize a diverse range of PAMPs. Briefly, TLR2, which works with TLR1 or TLR6, recognizes bacterial components, such as lipopeptide and lipoprotein of gram-positive bacteria; specifically, the heterodimer TLR1/2 can recognize triacyl lipopeptide, while TLR2/6 recognizes diacyl

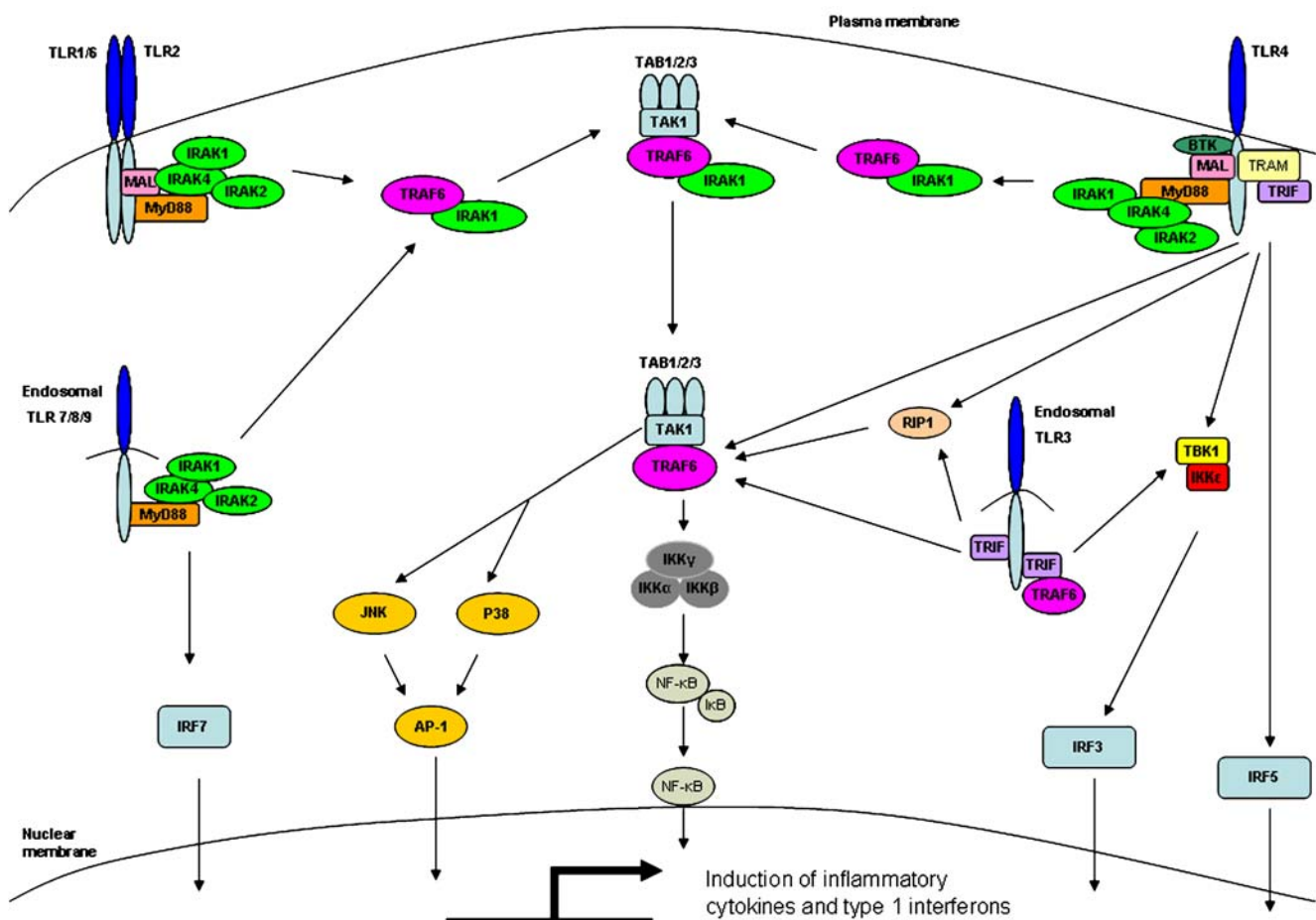
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lipopeptide. TLR3 recognizes double-stranded RNA (dsRNA). TLR4 recognizes lipopolysaccharide (LPS) of gram-negative bacteria, TLR5 recognizes bacterial flagellin, TLR7 recognizes synthetic imidazoquinoline-like molecules, guanosine analogs such as loxoribine, and single stranded RNA (ssRNA) from HIV-1, vesicular stomatitis virus (VSV) and influenza virus, human TLR8 recognizes imidazoquinolines and ssRNA while mouse TLR8 is thought to be nonfunctional. TLR9 recognizes bacterial and viral CpG DNA motifs and malaria pigment hemozoin [3]. The ligand for TLR10 remains unknown at present.

The recognition of PAMPs by TLRs triggers the activation of a signaling cascade, which culminates in the induction of genes involved in the inflammatory response (Fig. 1). After ligand binding, TLRs dimerize and undergo conformational changes, which are required for the recruitment of adaptor molecules, via their TIR domains. These adaptor molecules, namely, MyD88, Mal (MyD88 adapter-like)/TIRAP (TIR-domain-containing adaptor protein), TRIF (Toll-receptor-associated activator of interferon), TRAM (TRIF-related adaptor molecule), and SARM (sterile  $\alpha$  and armadillo motifs), contribute to the specificity of individual

responses to pathogens. Each TLR can mediate a tailored response in association with different combinations of these adaptors. Two major pathways can be activated by TLRs; the MyD88-dependent pathway results in the activation of NF- $\kappa$ B and activating protein-1 (AP-1), while the TRIF-dependent pathway results in the activation of type I interferons (IFNs) [4].

The MyD88-dependent pathway is used by all TLRs, except TLR3, and is also used by the type I IL-1 receptor (IL-1R1). Signaling is initiated by recruitment of MyD88 to TLRs, via TIR–TIR interactions [1]. MyD88 recruits IL-1 receptor kinase-4 (IRAK-4), allowing association and phosphorylation of IRAK-1, which in turn recruits and activates tumor necrosis factor receptor-associated factor 6 (TRAF6). TRAF6 activates TGF- $\beta$  activated kinase 1 (TAK1), which forms a complex with the TAK1 binding proteins, TAB1, TAB2, and TAB3. TAK1 then activates the IKK complex, which phosphorylates the I $\kappa$ B family and leads ultimately to the activation of NF- $\kappa$ B [5]. TAK1 also activates members of the MAP kinase family, which activate JNK, p38, and extracellular signal-related kinases (ERKs), leading to the activation of AP-1. The transcription



**Fig. 1** TLRs signal via different combinations of adaptors. All TLRs, except TLR3, use the MyD88-dependent pathway. TLR3 uses the TRIF-dependent pathway. TLR signaling results in the induction of inflammatory cytokines and type I interferons

factor IRF7 forms a complex with MyD88, IRAK1, and TRAF6 [6, 7]. Another transcription factor, IRF5, also associates in a trimeric complex with TRAF6 and MyD88 [8]. The IRF family of transcription factors are structurally related to NF- $\kappa$ B family members and have important roles in the regulation of type 1 IFN production. The translocation of these transcription factors, AP-1, NF- $\kappa$ B, and IRFs to the nucleus regulates the transcription, mRNA stability, and translation of numerous proinflammatory cytokine genes, such as TNF, IL-6, IL-12, and IFNs.

The TRIF-dependent pathway culminates in the induction of type I IFNs and proinflammatory signals. TLR3 and TLR4 make use of this pathway, and the adaptor molecule TRIF is essential for this pathway [9]. The adaptor molecule TRAM is essential for TLR-4 mediated TRIF-dependent pathway and appears to act as a bridging adaptor between TLR4 and TRIF [10]. TRIF interacts with TBK and IKK $\epsilon$ , which phosphorylate IRF3 and IRF7. Phosphorylated IRF3 and IRF7 form homodimers and translocate into the nucleus to induce IFN- $\alpha$  and IFN- $\beta$ . TRIF can also activate the NF- $\kappa$ B pathway in two possible ways; firstly, by recruiting TRAF6 via its TRAF6 binding domains in the N-terminal region, and secondly, the C-terminal region of TRIF contains a RIP homotypic interaction motif (RHIM), which mediates interaction with members of the receptor interacting protein (RIP) family, which also results in NF- $\kappa$ B activation [11]. Both of these pathways may converge at the IKK complex, resulting in maximum activation of NF- $\kappa$ B.

In the case of the other adaptors, Mal is necessary to recruit MyD88 to both TLR2 and TLR4 [12]. TRAM, as mentioned already, is used by TLR4 for the recruitment of TRIF and subsequent downstream signaling. The final adaptor molecule, SARM, has been described as a specific inhibitor of TRIF-dependent signaling [13].

TLR signaling needs to be tightly balanced and various negative regulatory mechanisms exist, ranging from extracellular decoy receptors, intracellular inhibitors, and membrane bound suppressors to degradation of TLRs and TLR-induced apoptosis. Because many TLRs are implicated in autoimmune, chronic inflammatory and infectious diseases, it is possible that some of these disease processes could involve overactivation of TLRs or dysregulated TLR signaling [14].

## NLRs

NLRs are reviewed separately in an article in this series so will only be discussed here briefly. The NLR family is a group of recently identified cytoplasmic PRRs that contain more than 23 members in humans. Although the ligands and functions of many of these receptors are still being identified, it is clear that their major role is to recognize cytoplasmic microbial PAMPs and/or endogenous danger signals and

initiate immunological responses. The NLR family includes NODs (nucleotide-binding oligomerization domain-1), NALPs (NACHT-, LRR- and pyrin-domain-containing proteins), IPAF (ICE-protease activating factor), NAIPs (neuronal apoptosis inhibitor factors), and CIITA [15].

The NLR family has a domain organization consisting of a C-terminal LRR domain, a central nucleotide-binding NACHT domain, and an N-terminal protein–protein interaction domain composed of a caspase recruitment domain (CARD), pyrin domain (PYD) or baculovirus ‘inhibitor of apoptosis’ repeat (BIR) domain [16]. NLRs can be grouped based on their effector domains; NODs and IPAF contain CARD effector domains, NALPs contain PYD domains, and NAIPs contain BIR domains.

While TLRs are associated with the plasma membrane or, in some instances, with lysosomal and/or endosomal vesicles, NLRs are thought to be localized to the cytosol. Recent evidence, however, shows that in epithelial cells, NOD2 contains molecular sequences that allow association with the plasma membrane [17]. Another recent study showed that NALP1 is located mainly in the nucleus whereas NALP3 is predominantly cytoplasmic [18]. There is also evidence that shows CIITA, another NLR, can be located in the nucleus [19].

Several NLRs are capable of detecting bacterial components, although the mechanisms for these are not clearly understood. NOD1 and NOD2 were the first NLRs reported to have a direct function as PRRs, in the recognition of peptides that are derived from the degradation of peptidoglycan (PGN), a bacterial cell wall component. NOD1 senses  $\gamma$ -D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) [20, 21], while NOD2—senses muramyl dipeptide (MDP) [22, 23]. NOD2 functions more as a general sensor of bacteria because PGN from both gram-negative and gram-positive bacteria contain MDP, while NOD1 mainly senses products from gram-negative bacteria, as this is where iE-DAP is found.

When NODs detect PGN, they rapidly form oligomers, which lead to the recruitment of the receptor-interacting protein 2 (RIP2) kinase, through CARD–CARD interactions [24]. This complex of NOD1-RIP2 or NOD2-RIP2 then recruits the inhibitor of NF- $\kappa$ B kinase complex (IKK), leading to the activation of NF- $\kappa$ B. TRIP6 and CARD6 positively regulate NOD-1- and NOD-2-dependent signaling of NF- $\kappa$ B. NOD2 activation is also regulated by GRIM19, TAK1, and erbin [16]. Another outcome resulting from NOD1 and NOD2 activation is the MAPK pathway that leads to the activation of p38 and ERK. NOD1 signaling also results in the activation of JNK [25, 26].

Several NLRs, namely, NALP1, NALP2, NALP3, and IPAF, are involved in the assembly of cytosolic protein complexes, called inflammasomes. The stimulus for the activation of NALP1 is unknown; however, the mechanical

rupture of THP-1 cells is capable of initiating inflammasome assembly. It is thought that the LRRs of NALP1 may recognize a ‘danger signal’ [4]. Nalp1b (a murine NALP1 variant) becomes activated in response to anthrax lethal toxin [27]. Upon ligand recognition, the NALP1 inflammasome assembles and comprises NALP1, the adaptor protein ASC, caspase-1, and caspase-5. The NALP2/3 inflammasome contains NALP2 or NALP3, ASC, cardinal, and caspase-1. The NALP3 inflammasome is activated by ATP and hypotonic stress, which are thought to mimic endogenous danger signals. It has also been shown to recognize bacterial RNA, ATP, and uric acid crystals and the antiviral imidazoquinoline compounds, R837 and R848 [11, 28–31]. Most recently, key components of the inflammasome were shown to be present in human keratinocytes, and contact sensitizers (CS) like trinitro-chlorobenzene could induce NALP3 inflammasome activation. It was also demonstrated that ASC- and NALP3-deficient mice display an impaired response to CS, suggesting that CS act as danger signals that activate the inflammasome in the skin [32]. The IPAF inflammasome consists of IPAF, ASC, and caspase-1 and has been implicated in the detection of intracellular flagellin from *S. typhimurium* and *L. pneumophila* [30].

Caspase-1 is the central effector of the inflammasome and activation of this multimeric protein complex results in the cleavage and activation of this cysteine protease. In turn, active caspase-1 cleaves and activates the proinflammatory cytokines IL-1 $\beta$  and IL-18. The inflammasome will be discussed in more detail below.

### TLR and NLR interplay

Aspects of NLRs and TLRs have been outlined individually above but it is emerging that they are connected at many levels. While they may not be dependent on each other to a full extent, it is clear that the links between them offer our immune system a more complex and integrated defense mechanism against invading microbes.

To begin with it is worth noting the similarities between the TLRs and NLRs, both in their structure and in the components of their signaling pathways. Firstly, both NLRs and TLRs sense ligands through their LRRs, and secondly, when IL-1 $\beta$  and IL-18 are activated by caspase-1 they signal through their respective receptors, namely, IL-1R1 and the IL-18 receptor. Like TLRs, these receptors possess an intracellular TIR domain and use MyD88 to mediate their intracellular signaling events responsible for activating NF- $\kappa$ B and other signaling cascades [33]. This highlights the fact that MyD88 is involved directly in the TLR pathway and indirectly in the NLR pathway.

Martinon and Tschopp highlight an interesting parallel between NLRs and the *Drosophila* Toll pathways. Both

pathways activate proteases that induce processing of cytokines that are involved in the activation of the TIR-containing receptors, IL-1R1 and Toll, respectively [34].

### TLRs and NODs

Many conflicting results have been reported about crosstalk between TLR and NOD signaling pathways. This is often due to contaminating features present in the ligands. For example, early reports showed that TLR2 was responsible for sensing PGN [35]; however, it emerged that this TLR2-mediated PGN sensing was due to impurities in the preparations and that PGN devoid of contaminating lipoproteins or lipoteichoic acid was not sensed by TLR2 [36].

Several groups have demonstrated that NOD1 and NOD2 are dispensable for normal TLR signaling [16, 25]. However, they may act in synergy with various TLRs in the production of cytokines to enhance the immune response [37–43]. For example, ieDAP, the NOD1 agonist, and MDP, the NOD2 agonist, both synergize with pure LPS, the TLR4 agonist to stimulate cytokine production [37].

It is interesting to note that it was shown that TLR2, TLR4 and NOD2 pathways are nonredundant recognition mechanisms of *Mycobacterium tuberculosis* that synergize for the induction of proinflammatory cytokines. This synergism was lost in individuals homozygous for the NOD2 3020insC mutation or macrophages harvested from TLR2 deficient mice [44].

Synergistic activity may not be the only link between these pathways since Watanabe et al. were able to demonstrate negative crosstalk between TLRs and NODs by showing that NOD2 negatively regulates TLR2-mediated Th-1 responses in a population of murine splenocytes [45]. However, these results were not corroborated when macrophages derived from bone marrow of NOD2 deficient mice were used [25].

In light of these data suggesting synergistic roles of NLR and TLR signaling followed by data suggesting antagonistic roles, it may be that synergism is specific to certain stimuli in certain cell types.

NLR responses are often increased upon TLR stimulation. For example, TNF or IFN- $\gamma$  treatment upregulates the expression of the NOD2 gene in intestinal epithelial cells and subsequently increases their LPS susceptibility. Similarly, Takehashi et al. found that NOD2 and NOD2 mRNA expression was increased by treatment with TNF, IL-1 $\beta$  or IL-6 in a macrophage cell line [46, 47].

At the protein level, NOD2 was shown to interact with TAK1 in the NOD2-mediated NF- $\kappa$ B pathway. The TAK1 complex is at the branch of MAPK activation in TLR signaling, and further investigation could determine if TAK1 has a role in TLR-NLR crosstalk [48, 49].

RIP2 is involved in NOD1 and NOD2 signaling, and initial reports suggested that RIP2 deficient macrophages displayed defective TLR signaling in response to LPS (TLR4 ligand), LTA (TLR2 ligand), and poly (IC) (TLR3 ligand) [50–52], indicating that RIP2 is involved in both TLR and NOD signaling. However, many preparations of bacterial components contain NOD1 and NOD2 stimulating molecules, which could explain the reduced TLR signaling observed in these macrophages [29]. Most recently, Park et al. used LPS preparations (that were devoid of any NOD1 and NOD2 activators) on RIP2 deficient macrophages, to show that RIP2 did not have a direct role in TLR signaling. Yet, they found that RIP2 is essential for the cooperation of NOD2 and TLR signaling in the production of cytokines when the amounts of their specific bacterial activators are limiting. So, although the pathways are independent, they do cooperate for optimal immune responses [29].

### TLRs and NALPs

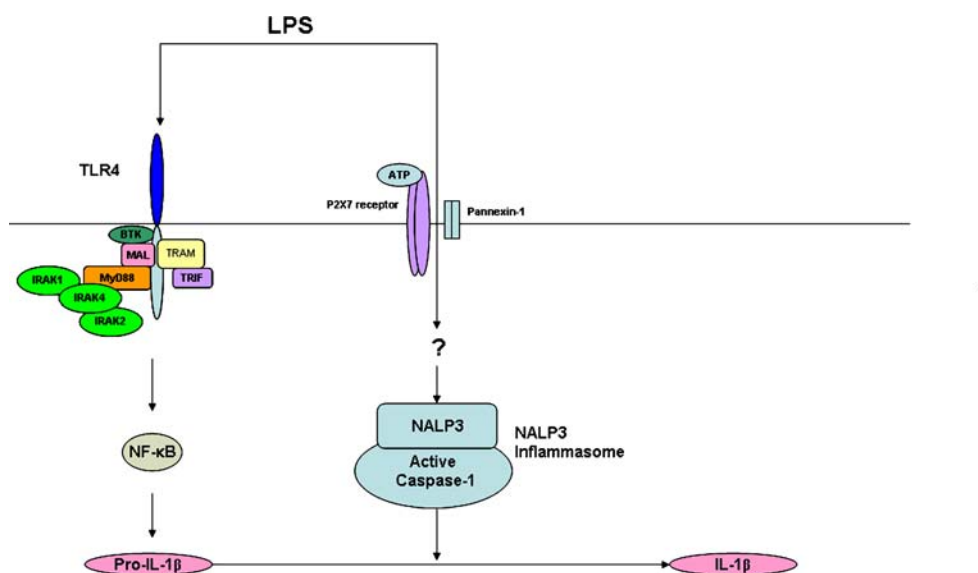
The most documented example of crosstalk between TLRs and NLRs is their joint involvement in the inflammasome pathway that leads to the secretion of mature IL-1 $\beta$  during microbial infection [53]. As described above, activation of TLRs by various ligands leads to NF- $\kappa$ B activation via the MyD88 adaptor, which is needed to induce the expression of the 31-kDa inactive precursor, pro-IL-1 $\beta$ , in the cytosol. This is referred to as ‘priming’ the system [54]. A 17-kDa biologically active form of IL-1 $\beta$  is generated by cleavage of the proform by caspase-1. Caspase-1 also cleaves pro-IL-18, which, unlike pro-IL-1 $\beta$ , is constitutively expressed. Caspase-1 itself is synthesized as an inactive 45-kDa zymogen that undergoes autocatalytic processing in re-

sponse to an appropriate stimulus. The active form of the enzyme comprises the p20 and p10 subunits, which assemble into a heterotetramer. Tschopp et al. discovered that caspase-1 forms the central component of the inflammasome, and the inflammasome acts as a platform for its activation [11].

The inflammasome activating stimulus is thought to be an agent that causes ionic perturbations, specifically potassium efflux. Loss of intracellular potassium results in the activation of calcium-independent phospholipase A<sub>2</sub> (iPLA<sub>2</sub>), and the inhibition of iPLA<sub>2</sub> prevents IL-1 $\beta$  processing (Fig. 2). The activation of the purinergic receptor P2X<sub>7</sub>, for which ATP is the main endogenous ligand, leads to the rapid opening of a potassium-selective channel, followed by the gradual opening of a larger pore and is mediated by a protein called pannexin-1 [55]. Activation results in potassium efflux along with plasma membrane depolarization, cell swelling, and disaggregation of the cytoskeletal network. P2X<sub>7</sub> knockout mice have been shown to have defective IL-1 $\beta$  release in response to ATP [56].

Kanneganti and colleagues investigated the role of pannexin-1 in NALP3 mediated caspase-1 activation [29]. Signaling through pannexin-1 was previously found to be crucial for caspase-1 activation and IL-1 $\beta$  secretion in LPS-stimulated macrophages pulsed with ATP or stimulated with the pore-forming toxins nigericin and maitotoxin [55]. Kanneganti and colleagues found that pannexin-1 is essential for P2X<sub>7</sub>-mediated activation of the NALP3 inflammasome by heat killed bacteria or LPS while infection with wild-type *Salmonella*, *Franciscella* or *Listeria* induced caspase-1 activation in the absence of ATP and independently of NALP3 and pannexin-1. *Salmonella* induces caspase-1 activation through IPAF and requires a bacterial type III or IV secretion system to deliver flagellin into the host

**Fig. 2** Crosstalk between TLR4 and NALP3. TLR4 responds to LPS and results in the activation of the NF- $\kappa$ B pathway and the production of pro-IL-1 $\beta$ . LPS stimulated macrophages pulsed with ATP signal through the P2X<sub>7</sub> receptor resulting in the activation of the NALP3 inflammasome and the activation of caspase-1. Caspase-1 then cleaves pro-IL-1 $\beta$  to produce active IL-1 $\beta$



cytosol [57]. *Franciscella* and *Listeria* also use the type III secretion system or pore-forming molecules to gain access to the host cytosol and to activate caspase-1, although the NLRs that they interact with are unknown at present.

Their results demonstrated that intracellular bacteria can induce caspase-1 activation in two ways. In the absence of ATP stimulation, a type III secretion apparatus or a pore-forming protein is required. In the presence of ATP, caspase-1 is activated through pannexin-1 and NALP3 even in the absence of a secretion system or pore-forming toxin [29].

Although TLR signaling is crucial for the upregulation of the IL-1 $\beta$  precursor, there has been some discussion as to whether the TLR pathway is directly involved in caspase-1 activation. An important consideration is the requirement of LPS to allow ATP to activate the inflammasome in priming macrophages. Either stimulus on their own is insufficient, and the mechanism appears to be TLR-independent because it is evident in MyD88 and TRIF knockout cells. PamCys and R848 have similar priming effects. Kanneganti and colleagues [29] found that NALP3-dependent caspase-1 activation induced by LPS is independent of TLR signaling, confirming that TLR signaling is not required for the activation of the NALP3 inflammasome, although it is still necessary for cytokine production (Fig. 2). These TLR-independent effects of LPS will require further analysis, both in the NALP3 inflammasome and in the other inflammasomes identified to date.

We have found what might be an important link between the adaptor protein Mal, used in TLR signaling, and the inflammasome component caspase-1. We showed that Mal is cleaved by caspase-1 and while Mal is not required for caspase-1 signaling, inhibition of caspase-1 activity impaired TLR2 and TLR4 signaling. During infection, bacterial products may be sensed, by TLRs and NLRs, which prime and activate the inflammasome complex. Active caspase-1 may then process Mal into its active form, allowing further signaling through TLR2 and TLR4 [58].

NALP6 and NALP12 have also been shown to activate the NF- $\kappa$ B pathway [59, 60]. However, others have reported that NALP12 dampens NF- $\kappa$ B signals, triggered by TLRs or TNF stimulation [61, 62]. For example, Williams et al. have shown that NALP12 is an antagonist of TLR-induced proinflammatory signals in myeloid cells. NALP12 binds IRAK1, resulting in the downregulation of its (IRAK1) activity in the TLR signaling cascade [62]. NALP3 was also once implicated in NF- $\kappa$ B activation but this was disproved using NALP3 deficient mice [29].

An example of stimulation of both TLRs and NLRs by pathogens is the ability of flagellin to stimulate both TLR5 and IPAF independently of each other, resulting in two different responses. TLR5 activates transcription factor NF- $\kappa$ B, resulting in the expression of pro-IL-1 $\beta$ . IPAF, initiates

IL-1 $\beta$  processing through caspase-1 and stimulates the secretion of mature IL-1 $\beta$ . By using two pathways for bacterial flagellin, the innate immune system may be able to modulate the intensity or quality of its response according to the virulence characteristics of the pathogen [57, 63]. Another example is that of R837 and R848, TLR7/8 ligands that have recently been shown to activate NALP3. R837 and R848 are used as immune response modifiers in the clinic and are being considered as vaccine adjuvants. The potent adjuvant, antiviral, and antitumor activities exerted by the imidazoquinoline compounds could be explained by their ability to activate both TLR and cryopyrin signaling [29].

## TLRs and diseases

Mutations in genes occur naturally during DNA replication but also as a result of damage to the DNA. Most new alleles are usually eliminated by genetic drift or remain in the population for a period as rare variants. If the DNA variant created by a mutation event becomes common in the population over many generations (found at a frequency of greater than 1%) it becomes known as a polymorphism. The simplest type of polymorphism, a single nucleotide polymorphism (SNP), results from a single base mutation. SNPs occurring in coding regions of genes may cause an alteration in the amino acid sequence and have a direct impact on protein function. However, a SNP located in a noncoding region may also be functionally important, for example, it may influence RNA splicing.

Polymorphisms in TLRs and their signaling molecules have revealed the importance of TLRs in human defense against diseases (Table 1), and many of these are inflammatory disorders, which are highlighted below.

Berdeli et al. found strong evidence for the biological role of TLR2 in acute rheumatic fever. They found that the common TLR2 Arg to Gln polymorphism at position 753 significantly contributes to the pathogenesis of the disease [64]. The R753Q SNP may also predispose people to staphylococcal infections or to tuberculosis [65], although a larger study came to the conclusion that this SNP is not associated with these infections [66]. Another SNP of TLR2—R677W—was found to be associated in Asian people with lepromatous leprosy [67].

Several groups have studied the D299G and T399I polymorphisms in TLR4 and have shown that these polymorphisms increase the risk of gram-negative infections [68]. A further study linked D299Q to an increased risk of systemic inflammatory syndrome [69] and increased susceptibility to late onset Alzheimer's disease [70]. These SNPs occur within the extracellular domain of TLR4, so the decrease in response is most likely mediated by a decrease in recognition of ligands. Inflammation has been implicated

**Table 1** SNPs in TLR genes associated with disease

Gene	SNP	Disease association
TLR2	R753Q	Acute rheumatic fever, staphylococcal infections, tuberculosis
	R677W	Lepromatous leprosy
	P631H	Meningitis
	16 934	Asthma
TLR4	D299G	Gram-negative infections, systemic inflammatory syndrome, late-onset Alzheimer's disease
	T399I	Gram-negative infections
	11 381	Prostate cancer
TLR5	392STOP	Legionnaires disease
TLR6	S249P	Asthma
TLR9	-1237	Asthma, Crohn's disease

as an etiological factor in several human cancers, and it is interesting to note that a SNP at position 11,381 in TLR4 was found to be more frequent among people with prostate cancer [71].

An association with a higher susceptibility to Legionnaires disease was shown for a common stop codon polymorphism (R392) in TLR5 [72]. SNPs in TLR6 and TLR10 have also been described and may possibly contribute to asthma susceptibility [73].

SNPs in genes involved in downstream TLR signaling pathways have also been described, e.g., NEMO, IRAK4, and I $\kappa$ B $\alpha$ . For example, anhidrotic ectodermal dysplasia with immune deficiency (EDA-ID) is a rare immunodeficiency characterized with infections by encapsulated pyrogenic bacteria, such as *Haemophilus influenzae*, *Streptococcus pneumoniae* or *Staphylococcus aureus*; viral illnesses caused by herpes simplex virus and cytomegalovirus; and fungal diseases such as *Pneumocystis jiroveci* pneumonitis [74]. Patients carry either X-linked recessive mutations in NF- $\kappa$ B essential modulator (NEMO) or autosomal dominant mutations in I $\kappa$ B $\alpha$ . These mutations result in impaired NF- $\kappa$ B activation and hence impaired TLR signaling [75]. Inherited IRAK4 deficiency is an autosomal recessive disorder mainly due to SNPs that encode stop codons at amino acid positions 287 and 293 of IRAK4 [76, 77]. IRAK4 is a crucial kinase in the TLR signaling pathway, as described earlier, and patients with IRAK4 deficiency are susceptible to invasive bacterial infections. Blood cells from these patients are impaired in their ability to make TNF $\alpha$  in response to activation by TLRs. It is interesting to note that IRAK4 deficient patients seem to improve with age, suggesting that adaptive immune responses compensate for the weakened innate responses. Bruton's tyrosine kinase (BTK) is a member of the Tec family of protein tyrosine kinases and is a component of the TLR signaling pathway. It interacts with the TIR domains of TLRs 4, 6, 8, and 9 and was also found to specifically associate with MyD88, Mal,

and IRAK1. Various mutations in the gene have been identified as being responsible for the X-linked agammaglobulinemia (XLA), an immune disorder characterized by a lack of circulating B lymphocytes. These include mutations that affect both the kinase activity of BTK and its ability to translocate to the plasma membrane [78].

However, sometimes SNPs in TLRs can have positive effects. For example, a recent study carried out found a SNP in the adaptor molecule Mal (S180L). This polymorphism is associated with a protective effect against infectious diseases, such as malaria, tuberculosis, and pneumococcal bacteremia [79]. The D299G SNP in TLR4 has also been associated with reduced risk in susceptibility to carotid artery atherosclerosis [80]. This mutation is also correlated with a lower rate of acute allograft rejection after transplantation [81], and a reduced prevalence of diabetic neuropathy in type 2 diabetes [82].

It is interesting to note that many pathogens are capable of exploiting the TLR system to evade the immune system. The  $\alpha$ - and  $\epsilon$ -Proteobacteria, which include the pathogens *Campylobacter jejuni*, *Helicobacter pylori*, and *Bartonella bacilliformis*, require flagellar motility to efficiently infect mammalian hosts. However, these bacteria possess specific changes in the TLR5 recognition site on flagellin that destroy TLR5 recognition. They have compensatory amino acid changes in their flagellin molecules that preserve their motility [83, 84]. Another bacterium, *M. tuberculosis* survives in host macrophages by inhibiting IFN- $\gamma$ -mediated signaling in a TLR2- and MyD88-dependent manner [85].

## NLRs and diseases

Again reviewed in a separate article in this series, this aspect will only be touched on briefly. Mutations in the gene encoding NOD2 have been associated with a genetic predisposition to Crohn's disease. MDP recognition and subsequent signaling is impaired in monocytes from Crohn's disease patients. Other diseases associated with polymorphisms in NLRs include atopic disease and asthma. Other rare autoinflammatory diseases involving NLRs include Blau syndrome, CINCA, Muckle–Wells syndrome, familial cold urticaria, and early-onset sarcoidosis [86].

## IL-1 $\beta$

An important part of the innate immune system is the secretion of IL-1 $\beta$ . As highlighted in this review, there is abundant evidence to convince us that there is interplay in the TLR and NLR pathways in the production of this cytokine, which is a major mediator of inflammation. IL-1 $\beta$  is a key mediator of the body's response to microbial

invasion and affects a large range of cells and organs; it evokes fever, hypotension, and the production of other cytokines, chemokines, and adhesion molecules. Dysregulated activation and release of IL-1 $\beta$  can lead to various inflammatory disorders [87]. Further understanding the mechanisms by which IL-1 $\beta$  is activated and controlled will involve deciphering the contributions of the TLR and NLR pathways. Ultimately, this will allow the development of therapies to help control chronic inflammatory disorders.

Another important consideration is that TLRs play a crucial role in linking innate and adaptive immune responses, through their actions on dendritic cells (DCs). Upon signaling through TLRs, they undergo a complex maturation process, involving upregulation of MHC molecules and coreceptors that enhance the ability of DCs to activate T cells. Type I IFN production (described above) is also thought to be a central molecule in the adaptive immune response. One of the next challenges will be to establish a better understanding of the complex interplay between TLR and NLRs and how this affects innate responses and the induction of adaptive immunity to a given pathogen.

## Conclusions

Recognition of pathogens by the innate immune system relies on specialized receptors to detect them, of which TLRs and NLRs are receiving much attention. Engagement of TLR signaling leads to the production of a variety of inflammatory cytokines. Members of the NLR family are also important in the production of cytokines and in the formation of a multiprotein inflammatory complex, the inflammasome. The view is emerging that the activation of both TLRs and NLRs is important to mount an efficient inflammatory response in the host. The best example of this is in the production of active IL-1 $\beta$ . Future work in this area will involve further delineation of the TLR and NLR signaling pathways and determining the precise mechanism of how they interact.

Continued research is needed to determine further understanding of the roles of TLRs and NLRs in inflammatory diseases. We need to apply this knowledge to further comprehend disease phenotypes and to develop effective therapies to combat these diseases. The association of members of the TLR and NLR families in various human diseases has been described and recent studies have described the genetic association of polymorphisms in genes encoding TLRs and NLRs or their downstream signaling molecules. These polymorphisms have demonstrated that TLR and NLR signaling affects the progression and development of several human diseases [88].

Another important aspect to consider in the future of this field is the involvement of other receptors. The RIG-1-like

receptor family (RLR) is a family of PRRs that recognize viral nucleic acids and activate interferon-regulated factor (IRF) family members. They share similarities with the TLRs in relation to their signal activation (NF- $\kappa$ B and IRF3) and the genes they induce (Type I IFNs). They also share similarities with NLRs due to the presence of CARD domains. It is likely that RLRs integrate with TLRs and NLRs to provide comprehensive antiviral protection [4]. Other receptors on innate immune cells include the mannose receptors, C-type lectins such as Dectin-1, Fc receptors, and scavenger receptors, which are also involved in the modulation of innate immune responses [89]. Because pathogens have multiple PAMPs and because innate immune cells express multiple PRRs, it can be expected that crosstalk among the signaling pathways occurs. Therefore, it will be important to ascertain the interactions of various PRRs with TLRs and with each other in the understanding of host defense.

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