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Review Article

Review on Toll-Like Receptors and Their role in Immunity

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Abstract

Toll-like receptors (TLRs) are the best-characterized membrane-bound receptors in innate immune cells, including macrophages and dendritic cells. Upon recognition of specific ligands originating from pathogen and modified self-derived molecules, TLRs trigger intracellular signaling cascades that involve various adaptor proteins and enzymes, resulting in the generation of proinflammatory and antimicrobial responses through the activation of transcription factors such as nuclear factor-κB. TLR-dependent signaling pathways are tightly regulated during innate immune responses by a variety of negative regulators. Efforts to modulate these regulatory pathways and signaling molecules may result in the development of new therapeutic strategies through TLR-based therapy. This paper reviews the roles of TLRs in innate immunity and also emphasizes newly described regulation of TLR-dependent signaling pathways.

Keywords: Toll-like receptor; Signaling pathway; Innate immunity

Abbreviation: APCs: Antigen Presenting Cells; CPG-ODN; Cytosine-Phosphate-Guanosine Oligodinucleotide; DCs: Dendritic Cells; dsRNA: Double-Stranded RNA; INF: Interferon; IRAKs: Interleukin-1 Receptor Associated Kinases; LPS: Lipopolysaccharide; LRRs: Leucine-Rich Repeats; MAPK: Mitogen Activated Protein Kinases; MyD88: Myeloid Differentiation Primary Response Protein 88; NF-KB: Nuclear Factor-KB; PAMPs: Pathogen Associated Molecular Patterns; PGN: Peptidoglycan; PRRs: Pattern Recognition Receptors; RSV: Respiratory syncytial virus; TIRAP: Toll-Interleukin 1 Receptor (TIR) Domain Containing Adaptor protein; TLRs: Toll-Like Receptors

Introduction

The mammalian immune system consists of two different arms-innate and adaptive immunity- and cooperative interactions of these two arms are required for elimination of infective pathogens with the highest efficiency. The innate immune system is an evolutionarily conserved system that provides the first line of protection against invading microbial pathogens and is mediated by phagocytes such as macrophages and Dendritic Cells (DCs). These cells sense microbial infection, engulf them and induce inflammatory responses. In contrast, adaptive immunity is highly specific and long lasting and has an immunological memory, but is initially developed in late phases of infection [23].

Unlike adaptive immunity, which takes weeks to generate, innate immune responses are mobilized within hours of exposure to a pathogen. This is accomplished by germ line encoded receptors on the surface of cells of the innate immune system. These receptors are known as Pattern Recognition Receptors (PRRs), which identify molecules produced exclusively by bacteria and other pathogens known as 'Pathogen-Associated Molecular Patterns (PAMPs) [40].

PAMPs include various components of pathogens such as Lipopolysaccharides (LPS), peptidoglycans, flagellin, bacterial DNA and viral double stranded RNA that are shared by many pathogens but not expressed by hosts. PAMPs such as LPS and peptidoglycan are essential structural components of the bacterial cell wall, and mutations within them are deleterious to microbes. Hence, PAMPs are relatively resistant to mutation and are ideal pathogen recognition receptor ligands [1].

PRRs present on immune cells bind to PAMPs and discriminate between self and non-self. This is the basic concept of innate immunity. PRRs of PAMPs include secreted PRRs (such as LPS binding protein), cell-surface PRRs (such as CD14, the macrophage scavenger receptor and the mannose receptor), intracellular PRRs (such as double-stranded, RNA-activated protein kinase) and Toll-Like Receptors (TLRs) [5].

Toll-Like Receptors (TLRs) are germline-encoded pathogen recognition receptors expressed most prominently on or in Antigen-Presenting Cells (APCs) such as macrophages and Dendritic Cells (DCs). It is well known that activation of TLRs on APCs initiates a cascade of intracellular signaling events, resulting ultimately in enhancing antigen presentation, the production and release of inflammatory cytokines and up-regulation of adhesion and co stimulatory molecules on the cell surface of APCs as well as priming the adaptive immune system. There are at least 10 different TLRs, and

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each serve as a receptor for one or more specific microbial molecules [26].

The objectives of this paper are:

- To overview the role of different Toll-like receptors in the immune system
- To highlight recent advances in our understanding of innate immune recognition of PAMPs through TLR

Overview of TLRs

The term TOLL originally referred to a cell surface receptor governing dorsal or ventral orientation in the early Drosophila larva. It was later found to also play a crucial part in antifungal defense together with other antimicrobial peptides. Sequencing of the Drosophila genome revealed the existence of 9 peptides belonging to TOLL family. Although a function in host defense could so far only be attributed to some family members, it is assumed that each member is involved in the host's defense against pathogens. In the 1990s, the first mammalian proteins structurally related to *Drosophila* TOLL were identified, and are now called human TOLL-Like Receptor (TLR) 1 and 4 [45].

TOLL and TLR family proteins are characterized by the presence of an extracellular domain with leucine-rich repeats and an intracytoplasmic region containing a TOLL/interleukin-1 Receptor homology (TLR) domain critical to both Drosophila TOLL and mammalian TLR signaling, indicating that they share homologous signaling components. In fact, for each signaling step, homologous components have been described for the two systems, mammalian TLR and Drosophila TOLL [18] suggesting phylogenetic conservation over time.

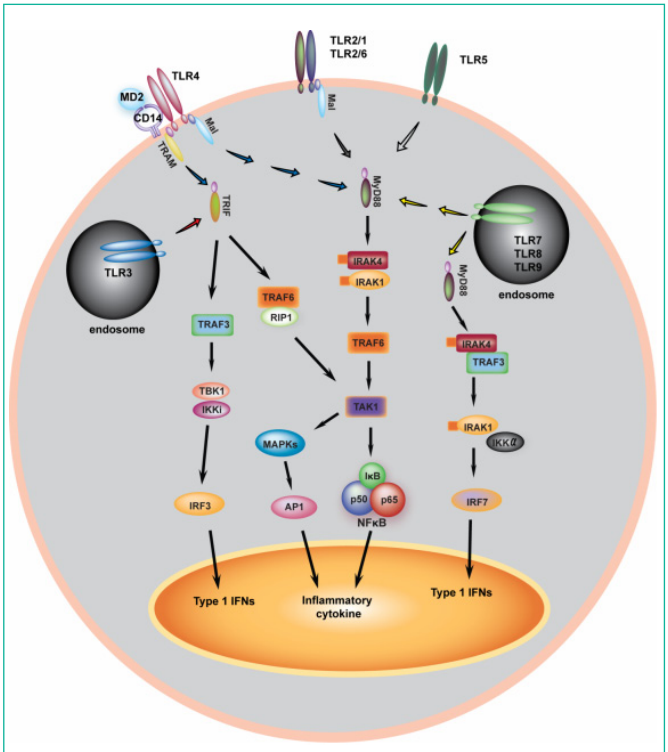

Figure 1: Toll-like receptor signaling pathways.

The TLR family members can be conveniently divided into two subpopulations with regard to their cellular localization. On the one hand, TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 are expressed exclusively on the cell surface and recognize microbial membrane components such as lipids, lipoproteins and proteins. On the other hand, TLR3, TLR7, TLR8 and TLR9 are localized in intracellular vesicles such as the endosome or lysosome and the Endoplasmic Reticulum (ER) and predominantly recognize microbial nucleic acid species [23].

TLRs and their Ligands

TLRs recognize the specific microbial patterns. Since the last decade there has been a steady increase in the number of TLR family members and their legands. Till now, ten TLRs have been identified and ligands have been known now for many of them. Most of the ligand studies are based on the knockout mice. Different TLRs seem to play crucial roles in the activation of the immune response to PAMPs. In spite of this specificity for the receptor ligand binding, the studies indicate that the overall innate immune response is the sum of signals generated by the interaction of multiple TLRs and other cooperating receptor molecules. For example, different TLRs can interact with the complex surface of the bacterium [5].

TLR1

TLR1, the first member of the TLR family, was identified by the presence of a domain homology found in both *Drosophila* Toll and human IL-1 receptors. TLR1 is expressed at higher levels in the spleen and peripheral blood cells. No direct ligands have been identified so far for TLR1, and its function remains unclear. TLR1 seems to act as a co-receptor. TLR1 was shown to associate with TLR2 in response to triacylated lipopeptides [47], but not diacylated lipopeptides. These observations indicate that TLR1 is able to discriminate among lipoproteins by recognizing the lipid configuration [43].

TLR2

TLR2 is involved in the recognition of multiple products of Gram-positive bacteria, mycobacteria and yeast. Earlier studies reported that TLR2 mediates LPS response, but later several studies indicated that TLR4 is the principal receptor for LPS. The cell wall of Gram-positive bacteria contains a thick layer of Peptidoglycan (PGN) within which lipoproteins and lipoteichoic acids are embedded, which can provoke immune responses similar to those generated by LPS. Analysis of TLR2-deficient mice demonstrated clearly that TLR2 is essential for the response to PGN [44].

TLR3

TLR3 recognizes double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Viral replication within infected cells results in the generation of dsRNA that can initiate antiviral defense; thus dsRNA can act as PAMPs. Stimulation with polyinosinepolycytidylic acid (poly (I: C)), a synthetic analogue of dsRNA, was shown to induce hyporesponsiveness in TLR3-deficient mice and marked responsiveness only in cells expressing TLR3, suggesting a specific recognition to poly (I: C) by TLR3. Furthermore, TLR3 signaling is not elicited by either single-stranded RNA (ssRNA) or dsDNA (Matsumoto *et al.*, 2002). TLR3 activation induces cytokine production through a signalling pathway dependent on MyD88 [2].

Table 1: Toll-like receptors and their ligands.

Toll-like receptors	Ligands	Pathogens
TLR1	Cofactor for TLR2	Gram-positive, Gram-negative bacteria, mycobacteria, spirochetes, mycoplasma
TLR2	Lipoteichoic acid, glycopeptides, peptidoglycans, zymosans	Gram-positive, Gram-negative bacteria, mycobacteria, soluble tuberculosis factor, LPS (some species), spirochetes, mycoplasma
TLR3	Poly I: C (double-stranded DNA)	Virus
TLR4	LPS (lipid A), respiratory syncytial virus (RSV) fusion protein, fibrinogen.	Gram-negative bacteria, RSV, plant
TLR5	Flagellin	Flagella of Gram-positive and Gram-negative bacteria
TLR6	Cofactor of TLR2	Gram-positive, Gram-negative bacteria, mycobacteria, spirochetes, mycoplasma
TLR7	Small antiviral compounds	?
TLR8	Small antiviral compounds	?
TLR9	Unmethylated CpG-DNA	Bacteria
TLR10	?	?

Source: Bhanu *et al.*, 2003

TLR4

TLR4 is the principal LPS receptor. LPS, a major component of the outer membrane of Gram-negative bacteria is composed of polysaccharides extending outward from the bacterial cell surface and a lipid portion, lipid A, which is embedded in the cell surface. LPS can provoke a variety of immune-stimulatory responses; for example, production of pro-inflammatory cytokines such as IL-12 and inflammatory effector substances such as nitric oxide [6].

Lipid A portion of LPS is mainly responsible for its biological activities. LPS can cause a clinically life-threatening condition called endotoxin shock. In addition to TLR4, a glycosylphosphatidylinositol anchoring protein, CD14, has been identified that facilitates LPS action by binding and retaining LPS on the cell surface [3].

TLR5

TLR5 recognizes flagellin from both Gram-positive and Gram-negative bacteria**.** Flagellin is the monomeric subunit of bacterial flagella. Flagellin shows potent pro-inflammatory activity by inducing expression of IL-8. TLR5 was identified by the presence of the TIR domain and is expressed in the spleen, peripheral blood leukocytes and epithelial cells. It has been found that the culture supernatants of the Gram-positive and Gram-negative bacteria have the ability to stimulate the Chinese hamster ovary cells expressing the human TLR5. It has also been confirmed that flagellated bacteria and not the non-flagellated ones activated TLR5, indicating that flagellin is the specific ligand for TLR5 [13].

TLR6

TLR6 is expressed in the spleen and peripheral blood leukocytes and, like TLR1, acts as a co receptor. Studies have shown that TLR6 cooperates with TLR2 to recognize PGN and the yeast cell-wall particle, zymosan**.** Furthermore, TLR6 and TLR2-deficient mice were reported to be hyporesponsive to mycoplasma MALP-2, a diacylated lipoprotein, suggesting that TLR2 and TLR6 coordinate the response to this ligand [34].

TLR7

TLR7 is abundantly expressed in the lung, placenta, spleen and peripheral blood leukocytes**.** TLR7 is phylogenetically close to TLR8 and TLR9, and has a higher molecular weight compared with hTLR1-6, largely as a result of a longer ectodomain. The natural ligand for TLR7 has not yet been identified. However, studies with TLR7-deficient mice have shown that TLR7 recognizes imidazoquinoline compounds such as R848, a small synthetic antiviral molecule [47].

TLR8

TLR8 was identified together with TLR7 and TLR9, and is expressed more abundantly in the peripheral blood leukocytes and the lung**.** The natural ligand for TLR8 is still unknown. Recently, human TLR8 and TLR7 were reported to independently confer responsiveness to R848, an imidazoquinoline compound with antiviral activity [21].

TLR9

TLR9, which is localized intracellularly, is involved in the recognition of specific unmethylated CpG-DNA sequences that distinguishes bacterial DNA from mammalian DNA. Bacterial DNA can stimulate immune cells. This activity is mainly because of the unmethylated CpG motifs, which are rarely detected in vertebrate DNA and, if present, are highly methylated. This stimulation leads to the production of Th1 (T helper 1) cytokines and co-stimulatory molecule up regulation. This feature of these CpG motifs makes them PAMPs. Analysis of TLR9- deficient mice has indicated that TLR9 is involved in recognizing this bacterial DNA as PAMP. All CpG DNA induced effects, including cytokine production, B-cell proliferation, dendritic cell maturation, and induction of systemic shock were completely abolished in TLR9- deficient cells and mice**.** Bacterial DNA should be exposed to the cell through digestion of bacteria in the phagoendosome before being recognized by the TLR9. This gives the endosome as a possible location for TLR9 [39].

TLR10

TLR10 is the last human member of the TLR family discovered so far, and its function and direct ligand are still unknown. Human TLR10 (hTLR10) is preferentially expressed on immune cells present in lymphoid tissues such as the spleen, lymph node, thymus, and tonsil. Phylogenetic analysis indicates that among all the human TLRs, hTLR10 is most closely related to hTLR1 and hTLR6; the overall amino acid identity is 50% and 49%, respectively [9].

TLR Signalling Pathway

The signaling pathways via TLRs originate from the cytoplasmic TIR domain. Furthermore, there are at least four TIR domain-containing adaptors (MyD88, TIRAP, TRIF, and TRAM) that have recently been shown to play important roles in TLR signaling.

These TIR domain containing adaptors are associated with TLRs through hemophilic interaction of TIR domains. Among these adaptors, myeloid differentiation protein (MyD88) was first characterized [24].

MyD88 Dependent Pathway

MyD88 contains a death domain in the N-terminus and a TIR domain in the C-terminus, thereby interacting with TLRs. When ligands bind to TLRs, MyD88 recruits IL-1 Receptor-Associated Kinases (IRAKs) to TLRs through interaction of the death domains of both molecules. IRAKs are activated by phosphorylation and then associate with TRAF6, leading to the activation of the IKB Kinase (IKK) complex. The IKK complex induces phosphorylation of IKB, which sequesters the transcription factor NF-KB in the cytoplasm. Degradation of IkB after phosphorylation triggers nuclear translocation of NF-KB to induce expression of inflammatory cytokine genes. MyD88-deficient mice showed no production of inflammatory cytokines such as TNF-α. IL-6, and IL-12, in response to all TLR ligands [28].

Thus, MyD88 is essential to signaling pathways of all TLR family members that lead to the production of inflammatory cytokines. IRAK harbors a death domain and a serine/threonine kinase domain.

MyD88 Independent Signaling Pathway

In accordance with the loss of inflammatory cytokine production, MyD88-deficient macrophages did not show any activation of NF-KB in response to ligands of TLR2, TLR7, and TLR9. However, stimulation with LPS (the TLR4 ligand) resulted in the activation of NF-KB in MyD88-deficient macrophages, although activation was delayed compared to wild-type macrophages. This finding indicates that although LPS-induced inflammatory cytokine production is mediated by the MyD88-dependent signaling pathway, a MyD88-independent component of TLR4-mediated signaling does exist [11].

A molecule termed as 'TIR Domain Containing Adapter Protein' (TIRAP) or 'MyD88 adapter-like' (Mal) has been found, which interacts with TLR4 and mediates MyD88-independent TLR4-signalling. Recently, interaction of TLR4 with its ligand was shown to induce the secretion of Antiviral Interferon-β (IFN-β) via a TIRAP/Mal-dependent, but MyD88-independent pathway. The downstream component of this MyD88 pathway remains to be elucidated [36].

Modulation of Immune Responses by TLRs

TLRs Bridge the Innate Immunity and Adaptive Immunity

Efficient immune responses depend upon a close interaction between the innate and adaptive immune systems. The innate immune system not only reacts promptly to microbial infection or environmental insult, but also instructs APCs to activate and secrete cytokines in order to polarize T cells towards an appropriate effector phenotype.

TLRs serve as an important link between the innate and adaptive immune responses. Different types of DCs selectively express cytokines, co-receptors and several other polarizing signals that promote the development of Th1, Th2, CD4+CD25+ Treg cells or the recently defined Th17 lineage, respectively. In this context, selected TLR ligands can be used alone or in combination as potential vaccine adjuvant to elicit the most appropriate immune response in humans or mice. The majority of known TLRs mediate the development of Th1-promoting DCs (type 1 DCs), whereas most of the PRRs mediate Th2-inducing DCs (type 2 DCs) [27].

Immature DCs express a full set of TLRs, which, on recognition of their ligands, induce DC maturation. Mature DCs express high levels of MHC and co-stimulatory molecules (CD80 and CD86) and migrate to draining lymph nodes where they present pathogen-derived antigens to naive T cells. TLRs also induce expression by DCs of various cytokines, including IL-12, which directs Th cell differentiation into Th1 effector cells. Only mature DCs will be able, through appropriate antigen presentation, to stimulate naïve T cells such that they differentiate into effector T cells. The types of effector T cells that evolve from the naïve cells are influenced greatly by the pattern of cytokines induced by the TLR engagement. Apparently, in addition to presenting antigens to naive T cells in an appropriate Major Histocompatibility Complex (MHC) context, the range of co-stimulatory signals delivered to T cells by DCs stimulated directly or indirectly by PRRs from pathogens mature into a specific form and are able to activate a single specific immune response that is appropriate for the elimination of the pathogen [25].

In this regard, DCs determine the nature of the foreign antigen and the intensity and phenotype of immune response generated. The development of different subtypes of effector T cell differentiation, a Th1, Th2 or Th17 immune response, is dependent upon the physical interaction between the activated status of the DCs and the naive T cells [49].

TLR signaling has been demonstrated to be involved in the immune recognition of allo- or xenografts and the occurrence of autoimmunity. This observation is supported strongly by the expression of TLRs on almost all immune cells and the identification of their endogenously expressing ligands by mammalian cells. Specificity of the TLRs for products of microbial origin allows them to detect the presence of infection and to induce activation of inflammatory and antimicrobial innate immune responses [26].

B lymphocytes play an essential role in adaptive immune response, but also primarily participate in innate immunity. LPS is a well-known strong stimulant of B cells. LPS stimulation activates B cells, leading to proliferation and IgM secretion.

B cells utilize two known receptor- signalling systems for LPS stimuli: RP105 (CD 180) and TLR4 [33]. RP105 is a type-I transmembrane protein of 105 kDa with a LRR motif in the extracellular domain, but without a TIR domain. RP105-mediated pathway is different from TLR4-mediated pathway. R P105 pathway is independent of MyD88 expression, which is essential to all the other known mammalian TLRs. RP105 receptor signalling pathway activates pathways similar to B Cell Receptor (BCR) signaling, such as the Lyn/CD19/Vav complex and the PI 3-kinase and signal some molecules. Thus, LPS signaling in B cells consists of two independent pathways, RP105-mediated pathway and TLR4- mediated pathway, and the B cell-specific LPS receptor RP105 shares signaling molecules with BCR to induce cellular activation.

This may explain why LPS-induced activation is impaired in B cells lacking various signaling molecules that are apparently not involved in the common TLR signaling pathway. This unique signaling system may be characteristic of B cells, which link innate immunity and adaptive immunity [48].

TLRs in Pathogen Recognition

TLRs in bacterial recognition

LPS is a potent innate immune stimulator constituting the outer membrane of Gram-negative bacteria [19]. TLR4 is involved in recognition of bacterial LPS. TLR4 forms a complex with another LRR protein known as MD-2 and this is mediated by ionic and hydrogen bonds in two oppositely charged patches [23,24]. There is no direct interaction between TLR4 and LPS, but MD-2 functions as the LPS-binding component in the TLR4–MD-2 complex [23].

TLR2 recognizes peptidoglycan and lipoteichoic acids, which are present in the cell membrane of Gram-positive bacteria. Accordingly, TLR2-deficient mice are highly sensitive to infection by the Gram-positive bacteria *Staphylococcus aureus*. In addition to Gram-positive bacterial components, TLR2 is involved in the recognition of several other bacterial components, such as lipoprotein/lipopeptides from a variety of bacteria, lipoarabinomannan from mycobacteria, a phenol-soluble modulin from *Staphylococcus epidermidis*, glycolipids from spirochetes *Treponema maltophilum*, and porins present in the outer membrane of Neisseria [30].

The mechanism by which TLR2 recognizes a variety of bacterial components can be partly represented by the association of TLR2 with TLR1 and TLR6. Expression of a dominant negative form of TLR6 in RAW264.7 macrophage cell lines inhibited TNF- production in response to TLR2 ligands, such as peptidoglycan and secreted modulin from Staphylococcus epidermidis. Macrophages from TLR6-deficient mice did not produce any TNF-in response to diacyllipopeptides from mycoplasma, but showed a normal response to triacyllipopeptides. In contrast, TLR1-deficient mice were impaired in triacyllipopeptides, but not diacyllipopeptides-induced TNF-β production. Thus, TLR1 and TLR6 cooperate functionally with TLR2, and participate in the discrimination of subtle structural differences among lipopeptides [34].

Bacterial components recognized by TLR2 and TLR4 are mainly present in the bacterial cell membrane. In addition to these components, flagellin, a protein component of the flagella extending out from the outer membrane of Gram-negative bacteria, has been shown to activate immune cells via TLR5 [13]. Furthermore, bacterial DNA can also serve as a ligand for TLRs. CpG DNA is characteristic of bacterial genomic DNA, in which unmethylated CpG motifs are present at an expected frequency. In mammalian genomic DNA, CpG motifs appear less frequently and are highly methylated, which causes no immunostimulatory activity. TLR9-deficient mice were unresponsive to CpG DNA, demonstrating that TLR9 is a receptor for CpG DNA [17].

TLRs in Fungal and Protozoal Recognition

Zymosan, a crude mixture of glucans, mannan, proteins, chitin, and glycolipids extracted from the cell membrane of fungi, activates immune cells via TLR2. The immunostimulatory activity of zymosan is seemingly attributed to the presence of glucan. Infection with the protozoan parasite *Trypanosoma Cruzi* causes Chagas diseases in human. Glycosylphosphatidylinositol (GPI) anchors that are present in the membrane of *T. cruzi* have also been shown to activate the innate immune cells via TLR2. Furthermore, host defense against *Leishmania major* and *Toxoplasma gondii* has been shown to be triggered by activation of TLR-mediated pathways [22].

TLRs in Viral Recognition

The first implication of TLRs in viral recognition was reported in vaccinia virus. The vaccinia virus genome encodes proteins that inhibit the TLR-mediated NF-B activation based on sequence homology with the TIR domain. Subsequently, TLR4 has been shown to recognize the fusion protein of respiratory syncytial virus (RSV). Accordingly, TLR4-mutated C3H/HeJ mice showed impaired ability to elicit inflammatory responses and clear the virus following RSV infection [14].

Furthermore, the envelope glycoprotein of Mouse Mammary Tumor Virus (MMTV) has been shown to activate B cells by interacting with TLR4 [35]. Thus, TLR4 presumably recognizes some viral-derived proteins. Double-stranded (ds) RNA is the most representative viral component that activates immune cells and induce type I interferons (IFN-β), which possess potent anti-viral activity. The synthetic dsRNA, polyinosinicpolycytidylic acid (poly (I: C)), has similar activity as dsRNA. Enforced expression of human TLR3 in HEK293 cells enabled the cells to activate NF-κB and the IFN-β promoter in response to dsRNA and poly (I: C) [2]. Furthermore, TLR3-deficient mice showed impaired responses to dsRNA and poly (I: C), indicating that TLR3 is involved in the recognition of dsRNA [2].

These findings indicate that viral recognition is mediated by TLR3 and TLR4, both of which have a unique pathway (MyD88-independent pathway). In addition to TLR3 and TLR4, TLR7 and TLR9 are seemingly responsible for viral recognition. Synthetic compounds, imidazoquinolines, induce potent anti-viral responses by eliciting the production of inflammatory cytokines, especially IFN-α. Indeed, one of the imidazoquinoline compounds, Imiquimod, is now clinically used for the treatment of genital warts caused by human papillomavirus infection. TLR7-deficient mice showed no responses to the imidazoquinolines, indicating that TLR7 is a receptor for the anti-viral compounds imidazoquinolines [16].

Although a natural ligand for TLR7 remains to be identified, TLR7 may also be involved in viral recognition. The TLR9 ligand, CpG DNA, also induces production of IFN-β from a certain type of dendritic cells (plasmacytoid DC), suggesting the possible involvement of TLR9 in anti-viral responses [15]. Consistent with this finding, herpes simplex virus-2 (HSV-2) has recently been shown to stimulate plasmacytoid DC to produce IFN-α via TLR9 [28].

TLRs in Endogenous Ligand Recognition

The possible involvement of TLRs, particularly TLR4, in the recognition of endogenous ligands has been demonstrated. For instance, TLR4 has been shown to recognize Extracellular Matrix (ECM) components, including the type III repeat extra domain A of fibronectin, oligosaccharides of hyaluronic acid, and polysaccharide fragments of heparin sulfate. TLR4 and TLR2 have been shown to be involved in the recognition of heat shock proteins, HSP60 and HSP70. TLR4 has further been shown to recognize fibrinogen [41]. Since all of these endogenous ligands are produced during inflammation, TLRs are presumably involved in inflammatory processes even in the

absence of infection. However, all of the endogenous ligands were used at very high concentrations to activate immune cells. In addition, the ability of HSP70 to activate macrophages has been attributed to contaminating LPS in the HSP70 preparation [12].

TLRs Variation in Domestic Animals

Bovine TLRs

As regard domestic animals, a lot of current work focuses on the TLR system of cattle. Recently, the bovine homologues of TLR2, TLR4, and MD-2 were cloned and partial sequences for boTLR3 and boTLR9 are available. The similarities at the amino acid level with the human and murine molecules are 77 and 67% for TLR2, 72 and 65% for TLR4, and 64 and 55% for MD-2, respectively. Using RT-PCR, mRNA for boTLR2 and boTLR4 could be detected in monocytes, Macrophages and DC. Thus resuming data on human cells, no differences in the amount of mRNA transcripts could be detected by a multiplex PCR for these TLR [32].

Chicken TLRs

Chicken TLRs repertoire consists of ten genes similar to that found in human and two fewer than mouse. Phylogenetic analyses show these to include six orthologs of mammals and fish, one fish ortholog and three unique to chicken [46]. Chicken TLRs 3, 4, 5 and 7 are directly orthologus to those found in other vertebrates. The chicken TLRs show a pattern of gene duplication and gene loss when compared to mammals. In particular, avian specific duplication of both TLR1 and TLR2 was observed. The duplicated genes, TLR2A and 2B found in chicken are both orthologs of the single TLR2 of mammals. Interestingly, there are remnants of second disrupted TLR2 like gene in tandem with functional TLR2 gene in mice and humans. Hence the duplication of TLR2 might have occurred prior to the divergence of mammals and birds and subsequently lost its functionality in mammalian lineage. Chicken TLR21 is an ortholog of TLR21 in fish and amphibians. It appears that TLRs 1LA, 1LB and 15 are unique to chicken. The chicken genome appears to miss a number of TLRs which are present in most mammals. The TLR7, 8 and 9 subfamily is present in fish and mammals but is only represented by TLR7 in chicken. TLR1 and 2 underwent gene duplication in chicken, whereas TLR8 and9 have been lost during the course of evolution. A wide set of TLRs are expressed by immune cells such as macrophages, dendritic cells and B cells as wells by non-immune cells such as epithelial cells which are located at the pathogen entry site [20].

Conclusion and Future Perspectives

The roles of TLRs in innate immunity and inflammation have been well-characterized. Upon stimulation, TLRs initiate intracellular signaling cascades to activate proinflammatory and innate immune responses. Each TLR recognizes distinct PAMPs to produce unique outcomes. TLR signaling pathways are activated by several intracellular adaptors and kinases and are associated with the signaling components of MAPK pathways. NF-κB-mediated transcription is required for the induction of the proinflammatory cytokines and mediators that contribute to innate and adaptive immunity. The diverse signaling pathways that cross-talk with TLRs and NF-κB are being progressively unraveled. A number of animal and clinical studies have revealed that TLR signaling pathways play a key role in innate immunity and host defense against pathogenic microbes. Recent insights into the function of several molecules involved in the negative regulation of TLR signaling have extended our understanding of the inhibitory feedback mechanisms through which a variety of extracellular and intracellular decoys fine-tune the activation of innate immune responses. TLR signaling plays a role in the pathogenesis of numerous human diseases; thus, therapies targeting TLR signaling are being developed. Understanding the roles of TLRs and their regulators in animals and humans will facilitate the development of novel therapeutics for TLR-mediated diseases. The identification and functional characterization of TLRs in *Drosophila* and mammals have brought our understanding of the innate immune system to a new level. The role of the TLRs in host defense is so fundamental; it is likely that their function affects most aspects of the mammalian immune system. The importance of the TLRs in the control of adaptive immune responses also makes them crucial targets for immune intervention.

References

1. Akira S, Hemmi H. Recognition of pathogen-associated molecular Patterns by TLR family. Immunol Lett. 2003; 85: 85–95.
2. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature. 2001; 413: 732-738.
3. Arbour NC. Toll-like receptors and their role in innate immunity. Nature Genet. 2000; 25: 187–191.
4. Banchereau J, Briere F, Caux C. Immunobiology of dendritic cells. Annu Rev Immunol. 2000; 18: 767–811.
5. Bhanu P, Singh RS, Chauhan Lokesh K, Singhal. Toll-like receptors and their role in innate immunity. current science. 2003; 85: 8- 25.
6. Bianchi ME. DAMPs, PAMPs and alarmins, all we need to know about danger. J Leukoc Biol. 2007; 81: 1–5.
7. Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. Regulatory T cells selectively express Toll-like receptors and are activated by lipopolysaccharide. J Exp Med. 2003; 197: 403–411.
8. Carty M, Bowie AG. Evaluating the role of Toll-like receptors in diseases of the central nervous system. Biochem Pharmacol. 2011; 81: 825-837.
9. Chuang T. Phylogenetic analysis indicates that among all the human TLRs, hTLR10. Biochim Biophys Act. 2001; 1517: 161–167.
10. Cohen J. The immunopathogenesis of sepsis. Nature 200 Manavalan, B., Basith, S., Choi, S. Similar Structures but different Roles - An Updated Perspective on TLR Structures. Front Physiol. 2011; 420: 885-891.
11. Doyle SE, Vaidya SA, O'Connell R, Dadgostar H, Dempsey PW. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity. 2002; 17: 251-263.
12. Gao B, Tsan MF. Endotoxin contamination in recombinant human Hsp70 preparation is responsible for the induction of TNF-3 release by murine macrophages. J Biol Chem. 2003; 278: 174-179.
13. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Goodlett DR. The innate immune response to bacterial flagellin is mediated by Toll-like receptor-5. Nature. 2001; 410: 1099-103.
14. Haynes LM, Moore DD, Kurt-Jones EA, Finberg RW, Anderson LJ, Tripp RA. Involvement of Toll-like receptor 4 in innate immunity to respiratory syncytial virus. J Virol. 2001; 75: 10730-10737.
15. Hemmi H, Kaisho T, Takeda K, Akira S. The roles of Toll-like receptor 9, MyD88, and DNA-PKcs in the effects of two distinct CpG DNAs on dendritic cell subsets. J Immunol. 2003; 170: 3059-3064.

16. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002; 3: 196-200.

17. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H. A Toll-like receptor recognizes bacterial DNA. Nature. 2000; 408: 740-745.

18. Horng T, Medzhtov R. TIRAP: an adaptor molecule in the TOLL signaling path way. Nat Immunol. 2001; 2: 835-841.

19. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y. Cutting edge: Toll like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lpsgene product. J Immunol. 1999; 162: 3749-3752.

20. Iqbal M, Philbin VJ, Smith AL. Expression patterns of chicken Toll-like receptor mRNA in tissues, immune cell subsets and cell lines. Veterinary Immunology and Immunopathology. 2005; 104: 117–127.

21. Jurk M. Toll-like receptor and their role in innate immunity. Nature Immunol. 2002; 3: 499.

22. Kataoka K, Muta T, Yamazaki S, Takeshige K. Implications for the recognition of fungi by innate immunity. J Biol Chem. 2002; 277: 36825-36831.

23. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. International Immunology. 2009; 21: 317–337.

24. Kiyoshi Takeda, Shizuo Akira. Microbial recognition by Toll-like receptors. Dermatological science. 2004; 34: 73-82.

25. Li X, Jiang S, Tapping RI. Toll-like receptor signaling in cell proliferation and survival. Cytokine. 2010; 49: 1-9.

26. Liu G, Zhao Y. Toll-like receptors and immune regulation: their direct and indirect modulation on regulatory CD4+ CD25+ T cells. Immunology. 2007; 122: 149–156.

27. Liu YJ. TSLP in epithelial cell and dendritic cell cross talk. Adv Immunol. 2009; 101: 1–25.

28. Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. J Exp Med. 2003; 198: 513-520.

29. Manavalan B, Basith S, Choi S. Similar Structures but Different Roles - An Updated Perspective on TLR Structures. Front Physiol. 2011; 2: 41.

30. Massari P, Henneke P, Ho Y, Latz E, Golenbock DT, Wetzler LM. Cutting edge: immunestimulation by Neisserialporins is Toll-like receptor 2 and MyD88 dependent. J Immunol. 2002; 168: 1533-1537.

31. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007; 449: 819-826.

32. Muzio M, Bosisio D, Polentarutti NM, D' Amico G, Stoppacciaro A, Mancinelli R, et al. Differential expression and regulation of TOLL-like receptors (TLR) in human leukocytes: selective expression of TLR3 in denderitic cells. J Immunol. 2000; 164: 5998-6004.

33. Ogata H. Toll-like receptors and their role in innate immunity. J Exp Med. 2000; 192: 23-29.

34. Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. Proc Natl Acad Sci USA. 2000; 97: 13766-13771.

35. Rassa JC, Meyers JL, Zhang Y, Kudaravalli R, Ross SR. Murine retroviruses activate B cells via interaction with Toll-like receptor 4. Proc. Natl Acad Sci USA. 2002; 99: 2281-2286.

36. Sakaguchi S, Negishi H, Asagiri M, Nakajima C, Mizutani T, Takaoka A. Essential role of IRF-3 in lipopolysaccharide-induced interferon-β gene expression and endotoxin shock. Biochem Biophys Res Commun. 2003; 306: 860-866.

37. Schwadner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by Toll-like receptor 2. J Biol Chem. 1999; 274: 17406-17409.

38. Shibolet O, Podolsky DK. TLRs in the Gut. IV. Negative regulation of Toll-like receptors and intestinal homeostasis: addition by subtraction. Am J Physiol Gastrointest Liver Physiol. 2007; 292: G1469-1473.

39. Shirota H. Toll-like receptors and their role in innate immunity. J. Immunol. 2001; 167: 66–74.

40. Singh BP, Chauhan RS, Singhal LK. Toll-like receptors and their role in innate immunity. Current Science. 2003; 85: 1156-1164.

41. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through Toll-like receptor 4. J Immunol. 2001; 167: 2887-2894.

42. Takeda K, Akira S. Toll-like receptors in innate immunity. IntImmunol. 2005; 17: 1-14.

43. Takeuchi O, Kawai T, Muhlradt PF, Radolf JD, Zychlinsky A, Takeda K. Discrimination of bacterial lipopeptides by Toll-like receptor 6. IntImmunol. 2001; 13: 933-940.

44. Takeuchi O. Toll-like receptors and their role in innate immunity. Immunity. 1999; 11: 443–451.

45. Tauszig S, Jouanguy E, Hoffmann JA, Imler JL. TOLL-related receptors and control of antimicrobial peptide expression in the Drosophila.proc. Nati1. Acad.sci. USA. 2000; 97: 10520-10525.

46. Temperley ND, Berlin S, Paton IR, Griffin DK, Burt DW. Evolution of the chicken Toll-like receptor gene family: a story of gene gain and gene loss. BMC Genomics. 2008; 9: 62.

47. Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T. Essential role of TIRAP/Mal for activation of the signaling cascade shared by TLR2 and TLR4. Nature. 2002; 420: 324-329.

48. Yazawa N. Toll-like receptors and their role in innate immunity. Blood. 2003; 11: 3573.

49. Van Vliet SJ, den Dunnen J, Gringhuis SI, Geijtenbeek TB, van Kooyk Y. Innate signaling and regulation of dendritic cell immunity. Curr Opin Immunol. 2007; 19: 435–440.