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Review Article

Toll-like receptors: Activation, signalling and transcriptional modulation

Dominic De Nardo *

Inflammation Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia
Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3050, Australia

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ABSTRACT

Families of innate immune receptors serve as the bodies primary defence system by recognising and rapidly responding to infection by microorganisms or to endogenous danger signals and initiating inflammatory processes. Whilst Toll-like receptors (TLRs) were the first family to be discovered, important and exciting discoveries continue to emerge into the molecular mechanisms that control their activation and regulation. Herein, I will provide an overview of TLR activation and their downstream signalling cascades, and discuss some of the recent findings concerning the assembly of a TLR oligomeric signalling platform, known as the Myddosome. Further, a brief examination of the importance of crosstalk between multiple TLRs or between TLRs and other innate immune receptors for appropriate and coordinated immune responses will be presented. Finally, I will discuss the importance of mechanisms that regulate TLRs with a focus on the role of activating transcription factor 3 (ATF3) in modulating transcriptional responses downstream of TLRs.

1. Introduction: Innate immunity driving acute inflammation

Inflammation is a key underlying mechanism of numerous physiological and disease processes [1]. Following microbial infection or tissue damage, inflammation is important for host protection via the destruction of invading pathogens and initiation of repair mechanisms ultimately designed to restore homeostasis. Innate immunity plays a key role in acute inflammation by acting as the primary host defence system to rapidly respond to microbial insults and tissue damage. To achieve these functions, families of highly conserved pattern recognition receptors (PRRs) have evolved to populate cellular membranes and cytosolic compartments of specialised immune cells (e.g. macrophages and dendritic cells) and monitor the local environment for signs of danger in the form of highly conserved microbial molecules present during infection. In addition to pathogen-derived danger signals, some of these receptors also drive sterile inflammation following recognition of endogenous molecules that may accumulate, become altered, or are released after cell death, tissue damage and metabolic dysfunction [2]. To date, the vertebrate innate immune system comprises plasma and endolysosomal membrane bound Toll-like receptors (TLRs), surface expressed C-type lectin receptors (CLRs), as well as cytosolic retinoic-acid-inducible gene I (RIG-I)-like receptors (RLRs), Nod-like receptors (NLRs), and several cytosolic DNA receptors including absence in melanoma 2 (AIM2) [3]. In some cases, members of different PRR families can recognise the same ligands at differing cellular locations; for instance, bacterial flagella can be recognised at the cell surface by TLR5, but can also be sensed in the cytosol via NLRC4 [4]. Such a system enables surveillance on multiple levels against an array of danger signals to ensure pathogens do not escape detection. Broadly, the recognition of external and intrinsic danger signals by PRRs culminates in the activation of specific transcription factors [5] or proteolytic pathways [6], resulting in the production of potent inflammatory mediators, including inflammatory cytokines, chemokines and interferons (IFNs). These factors mobilise host defences and facilitate acute inflammatory processes by coordinating recruitment of further immune cells and lymphocytes to sites of inflammation to ensure an appropriate inflammatory response to a specific insult is met. Signals emanating from the activation of PRRs are critical, not only for the clearance of invading microorganisms and the restoration of tissue homeostasis, but also for activation of the adaptive immune system.

The functional roles of many inflammatory mediators have long been known; however, the pathways leading to their production have only become clearer since the relatively recent emergence of the innate immune system, which began following the discovery of TLRs [7]. Although the roots of innate immunity date as far back as the landmark discoveries of phagocytes in the early 1900's by

* Address: Inflammation Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia. Tel.: +613 9345 2959; fax: +613 9347 0852.
E-mail address: denardo.d@wehi.edu.au

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Elie Metchnikoff [8], the field only really gained momentum some 100 years later following several important findings in the fruit fly, *Drosophila Melanogaster*. These were the discovery of the Toll gene [9], and the observation that Toll was required for *Drosophila* defence against fungi and bacterial infection [10,11]. The mammalian homologues of Toll were soon uncovered (i.e. the TLRs) [12,13] before other families of PRRs emerged [14]. Since then our understanding in the field of innate immunology and its underlying molecular mechanisms have rapidly advanced, and with it came great appreciation of its critical importance in many pathological and physiological settings. Indeed, besides microbial infections, TLRs and other PRRs are implicated in a multitude of chronic inflammatory diseases, including atherosclerosis, rheumatoid arthritis, gout and autoimmune disease [15–18]. In addition, PRRs have been shown to be critical for maintaining some physiological environments, such as homeostasis between the host and their commensal microbiota [19]. TLRs were the first PRRs to be uncovered and are perhaps the most widely investigated; however, exciting discoveries into the molecular mechanisms controlling their activation and regulation continue to be made. Such findings are not only important for a more comprehensive understanding of how the innate immune system works, but are also imperative for the design of pharmacological agents to modulate these processes during disease.

2. TLRs and their activation

TLRs are type I transmembrane domain proteins with a tripartite structure: they consist of an amino (N)-terminal extracellular domain containing leucine rich repeats (LRRs) that folds into a characteristic horseshoe-like structure and is responsible for ligand recognition; a single transmembrane spanning region; and a carboxyl (C)-terminal globular cytoplasmic Toll/interleukin-1 (IL-1) receptor (TIR) signalling domain, named in reference to the high homology between the intracellular domains of these receptors [20]. Indeed, both TLRs and the IL-1 family receptors, IL-1R, ST2 and IL-18R are thought to initiate a similar intracellular signalling cascade upon activation via their respective TIR domains [21]. Currently, 10 human and 12 mouse TLRs have been identified that can be broadly subdivided into two groups based on either (i) their localisation to the plasma membrane and activation by microbial membrane lipids or bacterial proteins, or (ii) those activated by microbial nucleic acids from within acidified endolysosomal compartments (see Table 1). For instance, cell surface TLR4 responds to lipopolysaccharide (LPS), an extracellular component of gram-negative bacterial cell walls, whilst the appearance of viral ds-RNA within endosomes activates TLR3. The nucleic acid sensing TLRs have been shown to traffic from the endoplasmic reticulum to endolysosomes prior to activation, which appears to be facilitated by the expression of Unc-93 homologue B1 (UNC93B1) [22–24]. Compartmentalisation of the nucleic acid sensing TLRs is thought to be important for preventing autoimmune responses to self-nucleic acids [25]. Some of the numerous exogenous and endogenous TLR ligands identified are summarised in Table 1. In most cases, engagement of TLRs by their ligands causes dimerisation of the receptor [26,27]; however, the endosomal TLRs, TLR7, TLR8 and TLR9 have been shown to exist as preformed dimers [28,29]. Dimerisation of TLRs in the presence of ligand results in conformational changes within the TIR domains, stabilising the receptor complex and leading to recruitment of TIR domain-containing adaptors to initiate downstream signalling cascades. Besides diversification within LRR domains, other mechanisms exist to increase the array of ligands recognisable to TLRs, including formation of TLR heterodimers, as well as cooperatively with accessory proteins or co-receptors. This is perhaps best exemplified by the recognition of oxidised low-density lipoproteins and Amyloid-β by the scavenger receptor CD36, which initiates assembly of a trimeric complex composed of CD36 and a TLR4/TLR6 heterodimer [30]. Furthermore, TLR2 is able to heterodimerise with either TLR1 or TLR6 in order to recognise triacylated lipoproteins and diacylated lipoproteins, respectively [31,32]. It was also recently shown that TLR11/TLR12 heterodimers in mice are critical for recognising *Toxoplasma* profilin during *T. gondii* infection [33].

3. Signalling pathways downstream of TLRs

Activation of TLRs on membranes leads to the recruitment of cytosolic TIR domain-containing adaptors in order to connect the receptors to downstream effector proteins. Five adaptors have been identified to a play role in TLR signalling: MyD88, MAL (also known as TIRAP), TRIF, TRAM and SARM [34]. Broadly, these adaptors can trigger two main pathways that are dependent on either MyD88 or TRIF, which lead predominantly, but not exclusively, to the production of inflammatory cytokines, or type I IFNs (e.g. IFNα/β),

Table 1
TLR expression, localisation and ligands.

TLR	Species	Localisation	Microbial ligands	Endogenous ligands	Synthetic ligands
TLR1	Human and mouse	Plasma membrane	Triacyl lipoproteins	Unknown	Pam3CSK4
TLR2	Human and mouse	Plasma membrane	Lipoproteins, zymosan, mannan, peptidoglycan, lipoteichoic acid,	Versican	Pam2CSK4, Pam3CSK4
TLR3	Human and mouse	Endolysosomal membrane	Viral dsRNA	mRNA	PolyI:C, polyA:U
TLR4	Human and mouse	Plasma and endolysosomal membrane	LPS	Oxidised low-density lipoprotein, Amyloid-beta	Lipid A derivatives
TLR5	Human and mouse	Plasma membrane	Flagellin	Unknown	Recombinant flagellin
TLR6	Human and mouse	Plasma membrane	Diacyl lipoproteins, lipotechoic acid, zymosan	Oxidised low-density lipoprotein, Amyloid-beta, versican	Macrophage-activating lipopeptide 2, synthetic diacylated lipoproteins, Pam2CSK4
TLR7	Human and mouse	Endolysosomal membrane	Viral and bacterial ssRNA	Immune complexes, self RNA	Thiazoquinoline and imidazoquinoline compounds (e.g. R848, imiquimod)
TLR8	Human and mouse	Endolysosomal membrane	Viral and bacterial ssRNA	Immune complexes, self RNA	Thiazoquinoline and imidazoquinoline compounds (e.g. R848, imiquimod)
TLR9	Human and mouse	Endolysosomal membrane	Viral and bacterial CpG DNA, DNA:RNA hybrids	Chromatin IgG immune complexes, self DNA	Class A, B and C CpG oligodeoxynucleotides
TLR10	Human	Plasma membrane	Unknown	Unknown	Unknown
TLR11	Mouse	Endolysosomal membrane	Profilin and flagellin	Unknown	Unknown
TLR12	Mouse	Endolysosomal membrane	Profilin	Unknown	Unknown
TLR13	Mouse	Endolysosomal membrane	Bacterial 23S ribosomal RNA (rRNA)	Unknown	23S rRNA derived oligoribonucleotide

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