Abstract

Introduction

Inflammation is triggered when innate immune cells detect microbial infection or tissue damage. Surveillance mechanisms involve pattern recognition receptors on the cell surface and in the cytoplasm. Most pattern recognition receptors correspond to pathogen-associated molecular patterns or host-derived damage-associated molecular patterns by triggering activation of various transcription factors. Induction of cytokines promotes the activation and recruitment of leukocytes, which are critical for eliminating pathogens and host debris. In order to avoid immunopathology, the system is very tightly regulated by numerous molecules that limit the magnitude and duration of the inflammatory response. In this review, we present current knowledge on pathogen recognition through different pattern recognition receptors and the complex signalling pathways responsible for activation of inflammatory and antimicrobial responses.

Conclusion

There are still many unresolved questions, such as the exact nature of the molecular events leading to different immune receptor activation and also identity of some unknown ligands for the receptors. Unravelling of these will offer insight into what critical components might be targeted for better therapeutic benefits in inflammatory disorders.

Introduction

The role of inflammatory response is to combat infection and tissue injury. The innate immune system constitutes the first line of host defence during infection and therefore plays a very crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens. The adaptive immune system, on the other hand, is responsible for elimination of pathogens during the late phase of infection and in the generation of immunological memory. The innate immune response is mediated primarily by phagocytic cells and antigen-presenting cells (APCs), such as macrophages, and dendritic cells (DCs), and has been regarded as relatively non-specific, whereas the adaptive immune response is characterized by antigen-specific receptors on lymphocytes generated by clonal gene rearrangements.

Innate immune cells of tissues such as macrophages, fibroblasts and dendritic cells, as well as circulating leukocytes, recognize pathogen invasion or cellular damage with pattern recognition receptors (PRRs). These receptors detect pathogen-associated molecular patterns (PAMPs), such as pathogen-derived nucleic acids and cell wall components, fungal β-glucan, bacterial flagellin, and lipopolysaccharide (LPS) from Gram-negative bacteria. PRRs also recognize damage-associated molecular patterns (DAMPs), released from injured cells during apoptosis or necrosis. DAMPs include uric acid, ATP, and the DNA-binding nuclear protein HMGB1 and amyloid β fibrils.

Activated PRRs then initiate signalling cascades to trigger the release of factors that promote recruitment of leukocytes to the infected region.

In this review, we look into the inflammatory signalling response emanating from the recognition of microbial infection and cellular injury by PRRs.

Among PRRs, membrane-bound toll-like receptors (TLRs) play a central role in the initiation of immune response against pathogen invasion. However, other PRRs are also involved—including membrane-bound C-type lectin receptors (CLRs), cytosolic proteins such as NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs).

Among these receptor types, TLRs and RLRs are primarily important for the production of type I interferons (IFNs), whereas NLRs are known to regulate interleukin-1β (IL-1β) production through activation of caspase-1.

Discussion

Toll-like receptors

TLRs were the first PRRs to be identified. They are also the most well-characterized and recognize a wide range of PAMPs. TLRs are transmembrane proteins which comprise an ectodomain, which contains leucine-rich repeats that mediate the recognition of PAMPs, a transmembrane region, and cytosolic Toll-IL-1 receptor (TIR) domains that activate downstream signalling pathways. TLRs are expressed either on the cell surface or on intracellular vesicles. To date, 10 and 12 functional TLRs have been identified in human and mice, respectively. Each TLR detects distinct PAMPs from bacteria, viruses, fungi and parasites.

Upon recognition of respective PAMPs, TLRs recruit a specific set of adaptor molecules that have TIR domains, such as MyD88 and TRIF, and initiate downstream signalling pathways.

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events that lead to the secretion of inflammatory cytokines, type I IFNs, chemokines and antimicrobial peptides. These responses cause neutrophil recruitment, activation of macrophages and induction of IFN-stimulated genes, resulting in clearance of pathogens.

TLR1, TLR2, TLR4, TLR5 and TLR6 are localized on the cell surface and recognize microbial membrane components, whereas TLR3, TLR7, TLR8 and TLR9 are expressed in intracellular vesicles and recognize nucleic acids. However, TLR11 is expressed both on the cell surface and in intracellular compartments. TLR13 is also expressed in intracellular vesicles. Intracellular vesicles in immune cells, in which TLR3, TLR7, TLR8 and TLR9 are localized, include the endoplasmic reticulum (ER), endosomes and lysosomes. The intracellular localization helps TLRs to recognize nucleic acids delivered to the intracellular compartments after the uptake of pathogens by infected cells. Different PAMPs are being sensed by different TLRs (Table 1).

After recognizing the microbial PAMPs, the TLRs activate the signaling pathways that provide specific immunological responses. The responses initiated by individual TLRs depend on the recruitment of TIR domain-containing adaptor protein (e.g. MyD88, TRIF, TIRAP or TRAM). MyD88 relays signals culminating in NF-κB and MAP kinase activation and the induction of inflammatory cytokines. It is engaged by all TLRs (with the exception of TLR3). TLR3 and TLR4 use TRIF to activate an alternative pathway leading to the activation of NF-κB and IFN-3 and the induction of type I IFN and inflammatory cytokine productions. TLR2 and TLR4 use TIRAP as an additional adaptor to recruit MyD88. TRAM acts as a bridge between TLR4 and TRIF. TLR4 is the only TLR that recruits four adaptor proteins and activates two distinct signaling pathways: the ‘TRIF-dependent’ and ‘MyD88-dependent’ pathways. TLR4 initially recruits TIRAP and MyD88. TIRAP localizes to the plasma membrane, where it serves to bridge the interaction between MyD88 and TLR4 upon LPS engagement. MyD88 then recruits IRAKs, TRAF6 and the TAK1 complex, leading to activation of NF-κB and MAP kinases. TLR4 is then endocytosed and delivered to intracellular vesicles to form a complex with TRAM and TRIF, which then recruits TRAF3 and the protein kinases TBK1 and IκK, which phosphorylates IRF3, leading to type I IFN expression.

TLR2 signals through MyD88 in an IRF3 and IRF7 phosphorylation-dependent fashion. TLR5 also signals through MyD88 to induce inflammatory cytokine production. However, TLR5 also recruits TRIF, in addition to MyD88, which leads to the activation of NF-κB rather than IRF3.

TLR7 and TLR9 are expressed primarily in plasmacytoid DCs (pDCs), but signal also through MyD88. MyD88 forms a complex with TRAF3, TRAF6, IRAK1, IκKα and IRF7. Then, IRF7 is phosphorylated by IRAK1 and IκKα, which then translocates into the nucleus to regulate the expression of type I IFN.

Although TLRs play a central role in the initiation of immune responses against a number of pathogens, it has become apparent that PRRs other than TLRs are also involved in PAMP recognition and the control of innate immunity. These include membrane-bound CLRs, cytosolic proteins such as NLRs and RLRs.

**C-type lectin receptors**

CLRs are a large superfamily of membrane proteins comprising one or more C-type lectin-like domains, which largely elicit inflammatory responses by recognizing fungal and bacterial PAMPs. The term ‘C-type lectin’ was introduced to distinguish between Ca2+-dependent and Ca2+-independent carbohydrate-binding lectins. CLRs have at least one carbohydrate recognition domain, which is a compact structural module containing conserved residues and determines the carbohydrate specificity of the CLR. CLRs exist as both soluble and transmembrane proteins. The transmembrane CLRs function as PRRs, and can be divided into two groups: (1) CLRs belonging to the mannose receptor family and (2) CLRs including the DC-associated C-type lectin 1 and DC immunoreceptor subfamilies. CLRs are primarily expressed by DCs and interact with pathogens through the recognition of carbohydrate (mannose, fucose and glucan) structures. Mannose structures allows the recognition of viruses and fungi. Fucose structures more specifically recognize some bacteria and helminths, while glucan structures recognize some fungi and mycobacteria. Recognition by CLRs leads to the internalization of the pathogen, its degradation and subsequent antigen presentation.

CLR triggering by different pathogens and the ensuing underlying signalling cascade depend on carbohydrate-specific signalling pathways and the DC subset. There are two ways by which CLRs induce signalling pathways. (1) CLRs such as macrophage inducible C-type lectin (CleC4e), dectin 2 (CleC6A) and blood DC antigen 2 protein (BDCA2) induce signalling pathways through

### Table 1: Ligands of Toll-like receptors

<table>
<thead>
<tr>
<th>PAMP</th>
<th>TLR</th>
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<tr>
<td>LTA, PGN</td>
<td>TLR2</td>
</tr>
<tr>
<td>LPS</td>
<td>TLR4</td>
</tr>
<tr>
<td>Flagellin</td>
<td>TLR5</td>
</tr>
<tr>
<td>RNA (microbial)</td>
<td>TLR7</td>
</tr>
<tr>
<td>DNA (microbial)</td>
<td>TLR9</td>
</tr>
<tr>
<td>Hemozoin</td>
<td>TLR9</td>
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<tr>
<td>Proflin</td>
<td>TLR11</td>
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immunoreceptor tyrosine-based activation motif (ITAM) containing adaptor molecules, such as Fc receptor γ-chain (FcRy) or DAP12. (2) Other CLRs, such as dectin 1 and DC-specific ICAM3 grabbing non-integrin, induce signalling pathways through the activation of protein kinases or phosphatases that interact with their cytoplasmic domains. Many CLRs are known to induce signalling pathways that modulate TLR-induced gene expression at the transcription level in an NF-kB-dependent fashion.

**RIG-I-like receptors**

RLRs comprise a family of cytoplasmic proteins consisting of three members: RIG-I, MDA5 and LGP2. RIG-I and MDA5 consist of two N-terminal caspase-recruitment domains (CARDs), a DExD/H box RNA helicase domain and a C-terminal repressor domain (RD), whereas LGP2 lacks a CARD and appears to be a positive regulator of signalling by MDA5 and RIG-I.

RLRs recognize viral RNAs in the cytoplasm. RNA virus infection leads to the generation of dsRNA and RNAs with 5′-triphosphate ends in infected cells. The helicase domain and RD are important for the recognition of these RNAs, while the CARDs are essential for triggering intracellular signalling cascades. dsRNA is present in cells infected with dsRNA viruses as well as generated during the course of ssRNA virus replication. As host cells do not produce dsRNA, the innate immune system discriminates between host and viral RNAs by the presence of dsRNA. MDA5 is responsible for IFN production to dsRNA stimulation. Reciprocally, RIG-I is essential for IFN production in response to ssRNA with 5′-triphosphate ends.

RIG-I and MDA5 proteins discriminate the lengths of dsRNA. Small dsRNAs of up to 1 kb are recognized by RIG-I but not by MDA5. On the other hand, dsRNAs bigger than 2 kb can be recognized by MDA5.

RIG-I-mediated signalling is positively and negatively controlled by ubiquitination of RIG-I. The CARDs of RIG-I undergo lys63-linked ubiquitination by TRIM25, a ubiquitin E3 ligase. This ubiquitination is necessary for efficient activation of the RIG-I signalling pathway. RIG-I also undergoes ubiquitination by the ubiquitin ligase RNF125, which leads to its proteasomal degradation. Ubiquitination by RNF125 is considered to inhibit aberrant activation of RIG-I signalling.

In response to viral RNAs, RIG-I and MDA5 associate with an adapter protein designated as mitochondrial antiviral signalling (MAVS), also known as virus-induced signalling adapter (VISA) or CARD adapter-inducing IFN-b (CARDIF). MAVS contains a CARD in its N-terminus and shares homology with the first CARDs of RIG-I and MDA5 for homotypic CARD–CARD interaction. The interaction is followed by MAPK activation and transcription induction by IRF3, IRF7 and NF-kB. Many of the components found downstream from TRIF in TLR signalling also are engaged by MAVS. For example, the kinases TBK1 and IκKε mediate IRF3/7 activation and induction of type I IFN genes.

**NOD-like receptors**

NLRs are the family of cytosolic immune receptors characterized by the presence of two shared features, C-terminal leucine-rich repeats (LRRs) and a NACHT nucleotide-binding domain (NBD). The LRR domains are responsible for the recognition of PAMPs and protein–protein interactions, whereas the NBD domain binds ribonucleotides and regulates self-oligomerization.

Though the NLRs have common features, they differ in their N-terminal domains. These differences are used to classify the NLR protein members. The largest group has an N-terminal pyrin domain (PYD) and is therefore called ‘NLRP’. Another group, which has an N-terminal CARD, contains the proteins nucleotide-binding oligomerization domain-containing 1 (NOD1, also called NLRC1), NOD2 (NLRC2) as well as the NLR family, CARD domain-containing 4 (NLRC4, also known as IPAF). Other NLR family members also have an acidic trans-activation domain or a baculoviral inhibitory repeat-like domain, such as the NLR family, apoptosis inhibitory protein 5 (NAIP5).

NLRP1, NLRP3 and NLRC4 assemble multimolecular protein complexes called ‘inflammasomes’ in response to various activators, leading to the activation of inflammatory caspsases. Activated caspase-1 regulates the maturation of IL-1β and IL-18 cytokines. AIM2, also known as PYHIN4, not belonging to the NLRP family, but to a different protein family (PYHIN), also assembles an inflammasome.

Different inflammasomes are activated by different stimuli (Table 2).

Upon activation, the inflammasome oligomerizes and recruits pro-caspase-1 directly by CARD interaction (e.g. NLRP1 or NLRC4 inflammasomes) or indirectly via the adaptor protein apoptosis-associated speck-like protein (ASC). In the latter case, NLRP3 or AIM2, for example, interact with ASC via the homotypic interaction of their PYDs. ASC, in turn, interacts with pro-caspase-1 via their CARDs.

**Table 2: Stimuli for Inflammasome**

<table>
<thead>
<tr>
<th>Inflammasome</th>
<th>Stimulus</th>
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<tbody>
<tr>
<td>AIM2</td>
<td>Cytosolic DNA</td>
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<tr>
<td>NLRP1</td>
<td>Anthrax toxin</td>
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<tr>
<td>NLRP3</td>
<td>Nigericin</td>
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<tr>
<td>NLRP3</td>
<td>Silica</td>
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<td></td>
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<td>Group B strep</td>
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<td>Listeria mono</td>
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<td></td>
<td>Bacterial RNA</td>
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Caspase-1 in resting stage is present in a catalytically inactive pro-form. The formation of the inflammasome initiates autocatalytic activation of caspase-1, and then cleaves the zymogens (pro-IL-1β and pro-IL-18) to active IL-1β and IL-18.

NLRP1 differs from the other members of the NLR family in its domain organization. Like other members of the NLRP subgroup, it has an N-terminal PYD, followed by an NBD and an LRR region. However, in contrast to all other members, NLRP1 has a C-terminal extension consisting of a FIIND motif and a CARD.

NLRP3 (cryopyrin), the most studied of all inflammasomes, contains the NLR-typical elements LRR and NBD and an N-terminal PYD. It can also recruit the adaptor protein ASC by PYD interactions. Oligomerization of NLRP3 in response to a stimulus and subsequent recruitment of ASC can activate caspase-1. In order to oligomerize, NLRP3 further requires binding of ATP to its NBD element. Interestingly, only one large NLRP3 inflammasome is formed per cell primarily consisting of adaptor ASC. NLRP3 activation is a two-step process. The first signal leads to NF-kB activation, which upregulates pro-IL-1β and NLRP3 expression. The second signal is generated by the stimulus itself in the form of crystals, aggregated protein, ATP or bacterial toxins. The second signal causes either lysosomal damage or potassium efflux or ROS production, which eventually leads to the inflammasome activation. In Gram-negative bacteria, NLRP3 activation requires a third signal: ATP binding of its NBD domain, followed by an oligomerization event.

Pathologic implication
More than any other cytokine family, the IL-1 family of ligands and receptors is gaining importance due to its association with various acute and chronic inflammation. Of the IL-1 family, IL-1β is of prime importance these days. Several autoinflammatory diseases such as the Muckle–Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS) and neonatal onset multisystem inflammatory disease (NOMID) characterized by episodes of fever, localized inflammation and skin rashes are attributed to mutations in the CIASI gene encoding NLRP3 or in the NLRP3 promoter. The classic autoinflammatory diseases—familial Mediterranean fever (FMF), pyogenic arthritis (PAPA syndrome) and cryopyrin-associated periodic syndromes (CAPS) are also caused by aberrant IL-1β production. More common diseases such as cancer, gout, type II diabetes, atherosclerosis and rheumatoid arthritis (RA) are responsive to IL-1β neutralization. Anakinra, an IL-1 receptor antagonist, is the first known drug for treating RA. Anti-IL-1β antibodies are also being used for the treatment of these diseases.

Conclusion
There are still many unresolved questions, such as the exact nature of the molecular events leading to different immune receptor activation and also identity of some unknown ligands for the receptors. Unravelling of these will offer insight into what critical components might be targeted for better therapeutic benefits in inflammatory disorders. As more tools become available, the future of inflammation research will be more exciting.

Abbreviations list
APC, antigen-presenting cell; CAPS, cryopyrin-associated periodic syndromes; CARD, caspase-recruitment domain; CLR, C-type lectin receptor; DAMP, damage-associated molecular pattern; DC, dendritic cell; ER, endoplasmic reticulum; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; LRR, leucine-rich repeat; MWS, Muckle–Wells syndrome; NBD, nucleotide-binding domain; NLR, NOD-like receptor; NOMID, neonatal onset multisystem inflammatory disease; PAMP, pathogen-associated molecular pattern; pDC, plasmacytoid DC; PRR, pattern recognition receptor; PYD, pyrin domain; RA, rheumatoid arthritis; RD, repressor domain; RLR, RIG-I-like receptor; TIR, Toll-IL-1 receptor; TLR, toll-like receptor.

References
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