Chapter 1

Innate Immune Receptors

Natalia Muñoz-Wolf and Ed C. Lavelle

Abstract

For many years innate immunity was regarded as a relatively nonspecific set of mechanisms serving as a first line of defence to contain infections while the more refined adaptive immune response was developing. The discovery of pattern recognition receptors (PRRs) revolutionised the prevailing view of innate immunity, revealing its intimate connection with adaptive immunity and generation of effector and memory T- and B-cell responses. Among the PRRs, families of Toll-like receptors (TLRs), C-type lectin receptors (CLR), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) and nucleotide-binding domain, leucine-rich repeat-containing protein receptors (NLRs), along with a number of cytosolic DNA sensors and the family of absent in melanoma (AIM)-like receptors (ALRs), have been characterised. NLR sensors have been a particular focus of attention, and some NLRs have emerged as key orchestrators of the inflammatory response through the formation of large multiprotein complexes termed inflammasomes. However, several other functions not related to inflammasomes have also been described for NLRs. This chapter introduces the different families of PRRs, their signalling pathways, cross-regulation and their roles in immunosurveillance. The structure and function of NLRs is also discussed with particular focus on the non-inflammasome NLRs.

Key words Innate receptors, Toll-like receptors, NOD-like receptors, MyD88, Pattern-recognition receptors, Non-inflammasome NLRs

1 PRRs, Ancient Receptors and the Answer to a One Hundred-Year-Question

The host response to invading pathogens is an essential physiological response; hence, maintenance of an organism's integrity in the face of such challenges has been a driving force in evolution. Indeed evidence for a "defence system" can be traced back to prokaryotes [1].

Before the molecular era in immunology, the notion that the immune system had evolved to defend the host from invaders was already accepted. However, it took almost one century to identify the mechanisms underlying immune recognition. In 1884, Élie Metchnikoff observed that cells of the water flea Daphnia could engulf and destroy spores of a yeast-like fungus with "some sort of secretion". He named these cells phagocytes [2] and for the first time described three functions that we now recognise as key attributes of the innate immune system: swift detection of microbes,

Francesco Di Virgilio and Pablo Pelegrín (eds.), *NLR Proteins: Methods and Protocols*, Methods in Molecular Biology, vol. 1417, DOI 10.1007/978-1-4939-3566-6_1,

phagocytosis and antimicrobial activity. During the twentieth century, the research of Paul Ehrlich and later of Karl Landsteiner shifted the focus of attention from phagocytes to humoral immunity. In the early 1900s, Ehrlich proposed his "side-chain theory", anticipating the existence of a mechanism of immune recognition based on what was later described as the antigen–antibody interaction [3]. Then in 1933, Karl Landsteiner characterised the specificity of the antibody-antigen interaction opening the molecular era of immunology [4]. "The clonal selection theory of acquired immunity" was introduced by Frank M. Burnet. Burnet's theory explained how the specificity of antibodies was generated in the first place [5], becoming a central paradigm in immunology for nearly 50 years, bringing adaptive immunity to the centre of attention of the scientific community.

For many years adaptive immunity was the subject of intense research and the remarkable diversity of the adaptive receptors overshadowed innate immunity. The immune response was conceived as a two-compartment system in which the early innate response was seen as an unsophisticated array of mechanisms containing the infection, while the more complex adaptive response was being generated to finally eliminate the pathogen and give rise to immunological memory. However, this paradigm was unable to explain a very basic observation: how primitive organisms lacking the adaptive components were able to protect themselves and distinguish self from non-self?

Even though diversification of living creatures has led to a multiplicity of non-self recognition strategies, the key molecular principles of discrimination seem to be conserved among phyla [1]. These observations led to the idea that the templates for innate immunity have been conserved from primitive life forms to humans and that discrimination of self vs non-self and recognition of pathogens rely on phylogenetically ancient first-line sensors that recognise invariant non-self patterns. Clearly not all the encounters taking place within the course of a life cycle will pose a threat to the host. Hence, the onset of the immune response must also be tightly regulated and directed to specific targets that may put the host's integrity at risk and to avoid self-recognition. This implies that the recognition of the invader must precede the onset of any effector mechanism and also contribute to instruct the system to mount an appropriate response.

The contemporary view of the innate immune system was revolutionised in 1989 by Charles A. Janeway Jr.. In his monograph "Approaching the Asymptote? Evolution and Revolution in Immunology", Janeway Jr. introduced the concept of "pattern recognition receptors" (PRRs) [6] postulating that PRRs recognising microbial-derived products link innate and adaptive immunity by activating antigen-presenting cells (APCs) to provide the second signal required for T-cell activation and initiation of the adaptive

response. He proposed that, opposed to the adaptive receptors, PRRs were non-clonally distributed receptors encoded by single non-rearranging genes. The proposed function of PRRs was to recognise structural patterns in molecules found in microorganisms but not in multicellular organisms to efficiently differentiate noninfectious self from infectious non-self. Janeway postulated that these conserved "pathogen-associated molecular patterns" (PAMPs, now also referred to as MAMPs for "microbe-associated molecular patterns") recognised by PRRs should be the result of a specific metabolic pathway characteristic for the microorganisms like a carbohydrate or lipopolysaccharide absent from the host.

He also reasoned that the adaptive immune response required two signals for activation: ligation of the specific receptor on the surface of a T or B cell by the antigen and a second signal derived from the antigen-presenting cell later identified as costimulatory molecules [6]. Janeway's lab subsequently established that several components of bacteria, yeast, and viruses had the ability to enhance costimulatory activities for T cells [7] and also demonstrated that cis-presentation of both antigen and costimulators was needed for T-cell activation [8] linking innate and adaptive immunity.

Later on, Polly Matzinger challenged Janeway's theory introducing the "danger theory", suggesting that the main determinant of immune activation is not the origin of the antigen itself but the extent of damage. Consequently, instead of sensing PAMPs or MAMPs, the immune system would recognise danger-associated molecular patterns (DAMPs) that could be produced by the host itself [9]. Anything that can cause tissue damage, whether of microbial or nonmicrobial origin, can be sensed and will trigger an immune response, while if the stimulus does not pose any hazard to the host, even being a microbe, it will be "ignored".

While these theories introduced new concepts on how innate recognition contributes to self–non-self discrimination, the discovery of the first PRR, a member of the Toll-like receptors (TLRs) [10, 11], provided essential proof. The discovery of TLRs subsequently led to the characterisation of other families of innate immune receptors and their ligands, and later studies revealed DAMPs, including ATP, heparan sulphate, HMGB1 and S-100 proteins that can trigger immune responses upon ligation of innate receptors [12].

In addition to this essential role in sensing microbes and damage, innate immunity also regulates and directs the activation of the adaptive immune system through polarisation of antigen-presenting cells equipped with the germline-encoded PRRs, shaping the overall outcome of the response (Fig. 1). Haematopoietic cells, including dendritic cells, macrophages, and neutrophils, and even T and B cells, as well as non-haematopoietic cells such as epithelial cells, contribute to this host-defence system by expressing different arrays of PRRs.

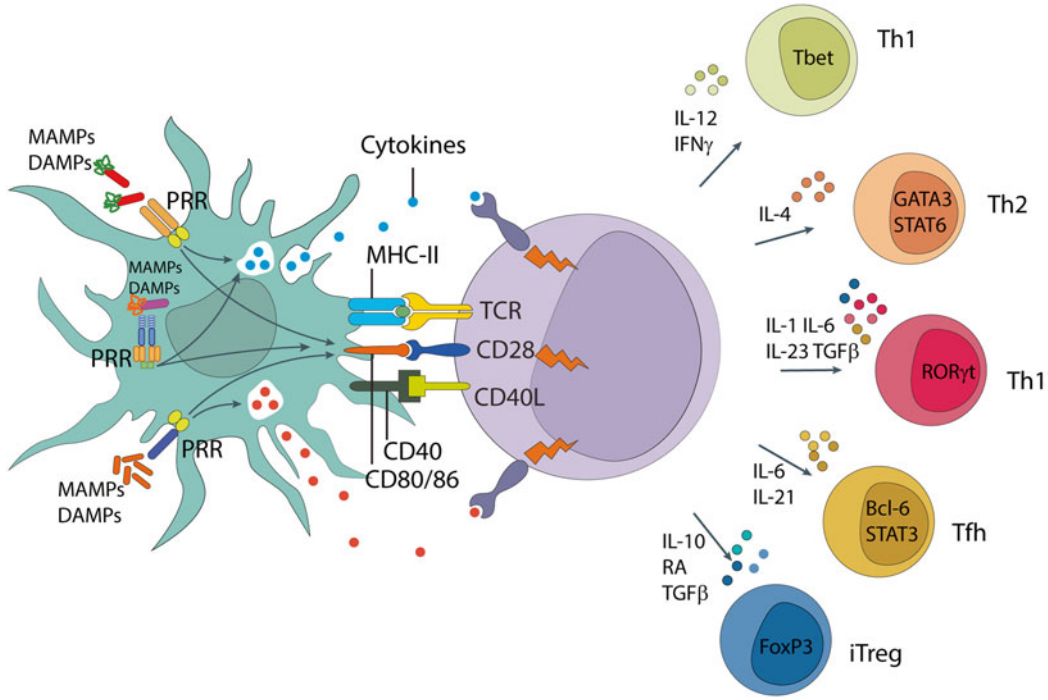

Fig. 1 Three-signal model of T-helper cell activation by antigen-presenting cells (APCs). APCs, typically dendritic cells (DC), sense microbial components (microbe-/danger-associated molecular pattern, MAMPs/DAMPs) through pattern recognition receptors (PRRs) triggering intracellular signalling cascades. This activates DCs, enhancing antigen uptake and processing for presentation in MHC class II molecules. Antigen-MHC-II complex constitutes signal 1 for the T-helper (Th) cell that interacts with it through its specific T-cell receptor (TCR). PAMP–PRR interaction also stimulates expression of costimulatory molecules on the APC, such as CD40, CD80 and CD86 that will constitute signal 2 for the Th cell. Signal 3 is given by the polarising cytokines and other various soluble or membrane-bound factors, such as interleukin (IL-) 12, interferon gamma (IFNγ), IL-4, IL-1, IL-6 IL-21, IL23, IL-10, tumour growth factor beta (TGFβ) or retinoic acid (RA). The specific combination of polarising cytokines promotes the development of Th1, Th2, Th17, T follicular helper cells (Tfh) or inducible T regulatory cells (iTreg). While the specific profile of T-cell-polarising factors is triggered by recognition of specific MAMPs and DAMPs by an array of PRRs, the interaction between CD40 on the APC and CD40-ligand (CD40L) expressed on the activated T cell contributes to stabilise the phenotype. *STAT* signal transducers and activators of transcription, *Tbet*: T-box transcription factor, *GATA:* globin transcription factor, *ROR:* RAR-related orphan receptors, *Bcl*: B-cell CLL/lymphoma 2, *FoxP3:* forkhead box P3

To date, along with TLRs, several other families of innate receptors have been characterised. PRRs can be subdivided into membrane-bound receptors that include TLRs, along with C-type lectin receptors (CLR), and cytoplasmic receptors including retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) and the nucleotide-binding domain, leucine-rich repeat-containing protein receptors (NLRs). A number of other PRRs including the cytosolic DNA sensor cGAS (cyclic GMP-AMP synthase) and the family of absent in melanoma (AIM)-like receptors (ALRs) have also been recently described [13].

When these receptors bind their agonists, they trigger an innate immune response by engaging certain signalling cascades that ultimately activate transcription factors such as nuclear factor kappa B (NFκB), activator protein-1 (AP-1), ETS domain-containing protein Elk-1, activating transcription factor 2 (ATF2), the phosphoprotein p53 and members of the interferon-regulatory factor (IRF) family, leading to specific gene expression programmes. Several of the genes being expressed encode chemokines, such as interleukin (IL)-8, CCL-2 and CXCL-1 that promote recruitment of leukocytes including neutrophils, monocytes and lymphocytes and a vast array of cytokines that will amplify the inflammatory response, enhance antigen presentation and costimulatory molecule expression, initiate tissue repair and direct T-cell polarisation and differentiation into different lineages of effector T cells (Th-1, Th-2, Th-17, regulatory T-cells (Tregs), among others) [14].

2 Toll-Like Receptors

The Toll-like receptors are the prototypical innate pattern recognition receptors that sense danger- and microbial-associated molecular patterns.

The first clues that linked TLRs to innate immunity came from studies carried out in the fruit fly *Drosophila melanogaster*. The founding member of the TLRs, the Toll protein, was initially identified as a gene product essential for the development of embryonic dorsoventral polarity in the fly [15]. Later, the protein Toll was shown to share homology with the previously identified interleukin-1 receptor 1 (IL-1R1) [16] through which the pleiotropic proinflammatory cytokine IL-1 exerts its effects [17]. The first striking finding was that, even though both proteins had dissimilar physiological functions, they contained similar amino acid sequences known to be essential for NFκB signalling [18], a factor originally described to mediate the response to lipopolysaccharide in B cells [19]. Finally, in 1996 the work of Bruno Lemaitre showed the involvement of the protein Toll in the antifungal response in *D. melanogaster* and production of the antifungal peptide drosomycin, confirming its role in innate immunity [10].

In 1997 the first human homolog for the *Drosophila* Toll protein was described by R. Medzhitov in Janeway's lab [11]. To date, 13 members of the TLR family have been identified in mammals including 10 human TLRs (TLR1–TLR10) and 12 murine TLRs (TLR1–TLR9 and TLR11–TLR13). Although most of the TLRs are conserved between humans and mice, TLR10 has lost its functionality in mice due to a retroviral insertion; TLR11, TLR12 and TLR13 are missing in the human genome [20]. Orthologs and paralogs for several mammalian TLRs have been also identified in different taxa including birds, amphibians, teleosts and agnathans.

In addition to insects, TLRs have been also traced back to ancient invertebrates including sponges, cnidarians, oligochaetes, molluscs and crustaceans [21].

2.1 Structure and Ligand Recognition in TLRs

Biochemically, TLRs are defined as a family of type-I transmembrane glycoproteins, typically composed of three domains: the N-terminal ectodomains, characterised by the presence of leucine-rich repeat (LRR) motifs which dictate ligand specificity, either by direct interaction or through accessory molecules, a hydrophobic transmembrane domain and the internal C-terminal domain that mediates intracellular signalling [22].

TLRs can be found either inserted in the cellular membrane or as membrane-bound proteins in endosomes. The Toll-like receptors 1, 2, 4, 5 and 6 are found primarily, but not exclusively, in the plasma membrane; conversely, TLR3, TLR7, TLR8, TLR9 and the murine TLR11, TLR12 and TLR13 are localised in intracellular endosomal and endolysosomal compartments [13]. Trafficking of TLRs is a tightly regulated process, and endosomal localisation normally requires UNC93B1, a transmembrane protein known to control the movement of TLRs from the endoplasmic reticulum where the assembly of TLRs takes place, to their final location in endosomes [23, 24].

The LRR portion of the TLR is responsible for ligand specificity [25, 26]. These ectodomains recognise a wide variety of biomolecules that can be derived from bacteria, fungi and parasites or endogenously generated (Table 1). The LRR is either extracellular or facing the luminal compartment of endosomes where they encounter molecules released by invading pathogens or damaged tissue. Typically they present a horseshoe form as described for other LRR-containing proteins [22]. However, the proposed crystallographic structures for several TLR–ligand complexes have revealed that, in contrast to what has been observed for most of LRR-containing proteins, ligand binding to the LRR portion of the TLRs occurs most often on the ascending lateral surface of the ectodomains [25, 27–29].

Comparative sequence analysis of the vertebrate LRRs grouped TLRs into six subfamilies, revealing that TLRs from different species grouped according the primary sequence of their ectodomains recognising similar types of ligands. This suggested that selective pressure to maintain specificity for certain ligands has dominated the evolution of the ectodomains. Among the mammalian subfamilies, the TLR1 subfamily containing TLR1, TLR2 and TLR6 is associated with recognition of lipoproteins and lipopeptides; the TLR3 subfamily recognises double-stranded RNA; the TLR4 subfamily is linked to recognition of lipopolysaccharides; the TLR5 subfamily recognises the structural protein of the bacterial flagellum, flagellin; and the TLR7 subfamily comprising TLR7–TLR9 recognises nucleic acids [30]. The TLR11 subfamily including

Table 1
Mouse and human TLR expression, ligands and pathogens recognised

TLR	Localisation	Species-specific expression	Natural ligands	Synthetic ligands	Type of pathogen recognised
TLR1	Extracellular	Human/mice	Triacyl lipopeptides	Pam3CSK4	Bacteria
TLR2	Extracellular	Human/mice	Lipoproteins, peptidoglycan, LTA, zymosan/mannan	Pam3CSK4	Bacteria
TLR3	Endosomal	Human/mice	dsRNA	polyI:C and polyU	Viruses
TLR4	Extracellular/ Endosomal	Human/mice	LPS, RSV, mannans and glycoinositolphosphate from *Trypanosoma* spp.	Lipid A derivatives	Gram-negative bacteria and viruses
TLR5	Extracellular	Human/mice	Flagellin	ND	Bacteria
TLR6	Extracellular	Human/mice	Diacylipopetides LTA and zymosan	MALP2	Bacteria
TLR7	Endosomal	Human/mice	ssRNA/short dsRNA	Imidazoquinolines and guanosine analogues	Viruses and bacteria
TLR8	Endosomal	Human/mice	ssRNA/short dsRNA	Imidazoquinolines and guanosine analogues	Viruses and bacteria
TLR9	Endosomal	Human/mice	CpG DNA hemozoin *Plasmodium* spp.	CpG ODNs	Bacteria, viruses and protozoan parasites
TLR10	ND	Human	ND	ND	ND
TLR11	Endosomal	Mice	Profilin/flagellin	ND	Apicomplexan parasites and bacteria
TLR12	Endosomal	Mice	Profilin	ND	Apicomplexan parasites
TLR13	Endosomal	Mice	Bacterial 23S rRNA	ND	Gram-negative and Gram-positive bacteria

ND not defined, *dsRNA* double-stranded RNA, *LPS* lipopolysaccharide, *LTA* lipoteichoic acid, *MALP2* macrophage-activating lipopeptide 2, *ODN* oligodeoxynucleotide, *polyI:C* polyinosinic–polycytidylic acid, *polyU* poly-uridine, *rRNA* ribosomal RNA, *RSV* respiratory syncytial virus, *ssRNA* single-stranded RNA, *TLR* Toll-like receptor

murine TLR11, TLR12 and TLR13 has been the least explored so far, probably because these receptors are absent in humans [31]. Their natural ligands have been identified only recently, revealing that similar to TLR5, TLR11 and TLR12 recognise proteins. Originally TLR11 and TLR12 were reported to recognise profilin, a protein derived from apicomplexan parasites like *Toxoplasma gondii* [32, 33]. Surprisingly a recent study reported that flagellin, previously reported as a ligand for TLR5, is also a ligand for TLR11 [34, 35]. Likewise, new studies revealed that TLR13 acts as a receptor for bacterial ribosomal RNA 23S [36].

Upon ligand binding, TLRs undergo a molecular rearrangement leading to the two extracellular domains forming an "m"-shaped homo- or heterodimer with the ligand staying in between the two receptors in a "sandwich-like" arrangement. This conformational change brings the transmembrane and cytoplasmic domains into close proximity, allowing the C-terminal TIR domains to generate an active interacting domain that triggers the intracellular signalling cascade. Recent studies have shown that the transmembrane domain (TMD) regions have a pivotal role during receptor oligomerisation. Strikingly, it was shown that isolated TMDs lacking the ectodomains and intracellular TIR domains replicate the homotypic and heterotypic interactions with the same partner receptors as the full length proteins, revealing the importance of this region for the interaction between TLRs [37].

The cytoplasmic signalling C-terminal domain presents homology to the IL-1R and is thus referred as the Toll-IL-1-resistance (TIR) domain. The TIR domain of the TLRs interacts with TIR-domain-containing adaptor molecules in the cytosol which in turn trigger downstream signalling pathways that lead to the expression of proinflammatory cytokines, chemokines, antiviral and antibacterial proteins, among others [38].

Notwithstanding the substantial progress on the structural characterisation of TLRs, more information is required to fully understand the interaction between each TLR and its proposed ligand. There are still no crystal structures available for several TLRs including mammalian TLR5 and TLRs 7–13. If ligand recognition is mediated by yet uncharacterised proteins bridging the interaction between the ligand and the LRRs, as is the case for TLR4 and LPS interaction, they will need to be elucidated. Finally, more information is needed to understand how TLR-TIR domains interact with each other or with the TIRs of adaptor molecules.

2.2 TLR Signalling Pathways

Typically, upon ligand recognition TLRs experience conformational changes that are critical for the recruitment of TIR-domain-containing proteins to the TIR domain of the receptor and transduction of the signal. There are five TIR-domain adaptor molecules: myeloid differentiation primary-response protein 88 (MyD88), MyD88-adaptor-like (MAL) also known as

TIR-associated protein (TIRAP), TIR-domain-containing adaptor protein-inducing IFN-β (TRIF) also named TIR-domain-containing molecule 1 (TICAM1) and TRIF-related adaptor molecule (TRAM) and sterile-α-and armadillo-motif-containing protein 1 (SARM1) [39].

MyD88 and TRIF act as switches for distinct signalling pathways that in turn activate two important families of transcription factors involved in regulation of several genes that are implicated in the control of the immune response. The MyD88 pathway ultimately, but not exclusively, leads to the nuclear translocation of the transcription factor NFκB, whereas the TRIF pathway mainly triggers translocation of the IRFs, particularly IRF3.

NFκB proteins regulate expression of a diverse array of genes involved in control of innate and adaptive immunity, cell cycle, anti-apoptotic response and stress responses. In the context of innate responses, NFκB has been implicated in the induction of genes encoding proinflammatory cytokines and leukocyte recruitment [40].

The family of IRF transcription factors plays important roles in cell growth, survival and differentiation of haematopoietic cells, a key function being the orchestration of antiviral responses through the induction of type-I interferons (IFN-I) [41].

In the past decade, several studies suggested that IRFs can also be activated in a MyD88-dependent fashion, and it is now widely accepted that the MyD88-IRF axis makes a major contribution to the immune response triggered by TLR activation. In the next sections MyD88-dependent and MyD88-independent signalling pathways are introduced followed by an overview of the role of IRFs in TLR signalling.

2.2.1 MyD88 Signalling Pathway

MyD88 is recruited by all TLRs except for TLR3, upon ligand recognition (Fig. 2). The first event in the MyD88 signalling pathway is the formation of a complex involving IL-1R-associated kinase (IRAK) members and MyD88 adaptor named the "myddosome" [42]. MyD88 associated with the cytoplasmic portion of TLRs interacts with IRAK members through homophilic interactions of the death domains. IRAK members associate with TRAF6, which in turn activates transforming growth factor-activated kinase 1 (TAK1). TAK1 then activates the I kappa B kinase (IKK) complex and mitogen-activated protein kinase (MAPK) pathway [43].

The IKK complex is the core element of the NFκB cascade, and it is essentially composed of two kinase subunits, IKKα and IKKβ, and a regulatory subunit, NEMO/IKKγ. NFκB is a family of transcription factors that while inactive, is kept in the cytosol through interaction with members of the IκB family. The TAK1 complex activates the IKK complex by phosphorylation, which in turn phosphorylates IκB proteins, allowing their ubiquitination and degradation by the proteasome. IκB degradation releases NFκB,

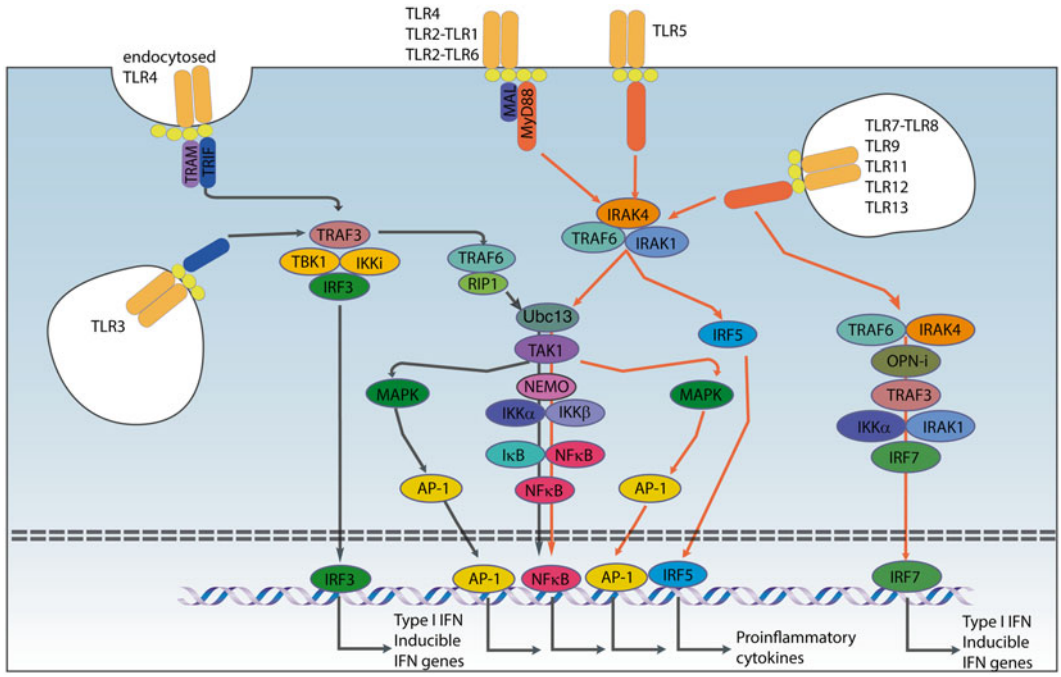

Fig. 2 TLR-activated signalling pathways. MyD88 associates with IL-1R-associated kinase (IRAK) members forming the myddosome. IRAK4 activates IRAK1, which in turn catalyses its autophosphorylation before it is released from the myddosome. IRAK1 then associates with TRAF6, an E3 ligase that together with UBC13 and UEV1A catalyses its own ubiquitination as well as ubiquitination of the transforming growth factor-activated kinase 1 (TAK1) protein complex formed by TAB1, TAB2 and TAB3. TAK1 then activates the I kappa B kinase (IKK) complex and mitogen-activated protein kinase (MAPK) pathway. The TAK1 complex activates the IKK complex by phosphorylating the IKKβ subunit. In turn, the active IKK complex phosphorylates IκB proteins which allows ubiquitination and degradation by the proteasome. As a result of IκB degradation, NFκB is released. Free NFκB translocates into the nucleus. Effector kinases of the MAPK pathway JNK, p38 and ERK are also activated, leading to AP-1 translocation into the nucleus to activate transcription of inflammatory genes. IRF5 can be also recruited to the MyD88–IRAK4–TRAF6 complex, phosphorylated and translocated to the nucleus to promote expression of proinflammatory cytokines. TLR4, TLR1/TLR2 and TLR2/TLR6 require recruitment of the adaptor MAL to activating the MyD88-dependent pathway. TRIF is recruited to TLR3 and endosomal TLR4. Endosomal TLR4 also requires recruitment of TRAM to initiate signalling. TRAF3 activates TBK1 and IKKi, which mediate phosphorylation of IRF3 triggering its dimerisation. IRF dimers translocate to the nucleus to induce expression of type-I IFN and IFN-inducible genes. TRIF also interacts with TRAF6 and RIP1, mediating NFκB activation. Endosomal TLRs sensing nucleic acids can activate the MyD88–TRAF6–IRF7 axis. Preferentially in plasmacytoid DCs, a complex consisting of MyD88–TRAF6–IRAK4–IRAK1–IRF7 is formed. OPN-i, TRAF3 and IKKα are also involved in this complex. Formation of the complex triggers IRF7 phosphorylation by IRAK1 and subsequent translocation to the nucleus to induce expression of type-I IFN and IFN-inducible genes

consisting of p65 (also known as RelA), c-Rel and p50 which translocates into the nucleus to activate transcription of cytokine genes associated with inflammation including TNF-α, IL-1β, IL-6 and IL-12p40; genes encoding cell adhesion and recruitment molecules like CXC and CC chemokines; and growth factors and antiapoptotic signals [44].

MAPK pathway activation results in the activation of the effector kinases c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK). Following TLR dimer formation in response to ligand recognition, activation of TRAF6 and TAK1 will activate the kinases MKK3 and MKK6, which will phosphorylate p38 and JNK, respectively. JNK phosphorylates c-Jun which binds to c-Fos to form the complex known as AP-1, which is then translocated into the nucleus to activate transcription of inflammatory genes [45].

Additionally, the p38 pathway can regulate gene expression through phosphorylation of the transcription factor cAMP response element-binding protein (CREB). TLR-dependent phosphorylation of CREB enhances its transactivation potential and plays an important role in regulating the transcriptional induction of many proinflammatory mediators, including cyclooxygenase 2 (COX-2) and TNF-α [46].

The role of the ERK pathway in TLR-induced responses has received less attention, but it is known to regulate gene expression at transcriptional and posttranscriptional levels. Another MAP3K known as Tpl2 is used instead of TAK1 to activate the ERK pathway downstream most of the TLRs [47].

The generation of MyD88 knockout mice and cell lines confirmed the crucial role of MyD88 in proinflammatory cytokine production and NFκB activation upon TLR ligation. Deficiency in the MyD88 signalling pathway resulted in impaired inflammatory cytokine secretion in response to several TLR agonists including the ligand for TLR2/TLR6, mycoplasmal macrophage-activating lipopeptide-2 (MALP-2) [48], CpG DNA which signals through TLR9 [49], the TLR5 ligand flagellin [50] and LPS and the ligand for TLR4 [51].

MyD88 deficiency also impaired cytokine secretion and NFκB activation in the response to IL-1β and IL-18, but not to TNF-α, IL-2 or IL-4 [51, 52]. While TNF-α, IL-2 and IL-4 signal through unrelated receptors, IL-1β, IL-18 and all the TLR ligands require different receptors of the TLR–IL-1R superfamily [53]. These observations suggested that MyD88 is a universal adaptor for this receptor superfamily. However, not all the effects induced by the TLR4 ligand LPS or the TLR3 ligand poly(I:C) were completely abrogated in MyD88 knockout mice, pointing to the existence of an alternative MyD88-independent signalling pathway that was exclusively activated upon engagement of TLR4 and TLR3.

2.2.2 TRIF-Dependent Pathway

Several observations contributed to the hypothesis that a MyD88-independent TLR signalling pathway existed. First, it was reported that LPS and poly(I:C) were able to induce dendritic cell maturation in MyD88$^{-/-}$ dendritic cells as revealed by upregulation of costimulatory molecules [51, 54]. Also, even though degradation of IκBα was delayed in MyD88$^{-/-}$ macrophages stimulated with

poly(I:C) or LPS, JNK and p38 were activated to a similar extent and with comparable kinetics to those seen in the wild-type cells [54]. Besides, despite the fact these cells failed to produce inflammatory cytokines, NFκB and MAPK were activated, albeit with delayed kinetics [55]. Moreover, these cells responded to TLR4 and TLR3 agonists by secreting IFN-β [56] and IP-10 (CXCL-10) [56]. TRIF was identified as the alternative adaptor molecule downstream of TLR4 and TLR3 [57–59]. Generation of TRIF knockout mice confirmed that TRIF was required for IFN-β production in cells stimulated with LPS or poly(I:C) and late activation of NFκB and MAPK was abolished in MyD88/TRIF knockout mice [58].

Interestingly, TRIF has the ability to trigger both IRF and NFκB translocation to the nucleus, activating type-I interferon (IFN-I) and interferon-inducible genes as well as transcription of inflammatory genes (Fig. 2). Amino and carboxy-terminal domains of TRIF have a different ability to bind proteins that act downstream in the signalling pathway. The C-terminal region interacts with receptor-interacting protein 1 (RIP1) kinase through its RHIM (RIP homotypic interaction motif), which after ubiquitination forms a complex with TRAF6 and TAK1. Ultimately, the formation of this complex will activate TAK1 and result in NFκB and MAPK activation, but not IFN-β secretion [60]. On the other hand, the N-terminal domain recruits the noncanonical IKKs TBK1 and IKKi and TRAF3, leading to activation of IRF3, which after forming a dimer translocates into the nucleus to induce transcription of IFN-I genes including IFN-β. The N-terminal domain can also recruit TRAF6, leading to nuclear translocation of NFκB and proinflammatory cytokine secretion [60].

TLR4 is unique in its capacity to activate both the MyD88 and TRIF pathways and entails the most complex signalling machinery of all TLRs. TLR4 uses the adaptor protein MAL as a bridge between the receptor and MyD88. MAL is also used by TLR2 although to a lesser extent. The adaptor TRAM links TRIF to TLR4 to induce IRF3 signalling. Subcellular localisation seems to be a critical factor for the activation of the TRIF pathway; indeed all TLRs activating this pathway are localised in endosomes. For the particular case of TLR4 upon activation, the receptor is endocytosed in endosomes. Change in its subcellular localisation acts as a switch between the MAL/MyD88 and the TRAM/TRIF signalling pathways, wich are activated sequentially rather than simultaneoulsly. [39]. SARM has been shown to be another important TIR-adaptor protein involved in the regulation of the TRIF pathway. However, in contrast to the other TIR-adaptor molecules, SARM acts as a negative regulator of TRIF [61].

2.2.3 IRF and Myd88

Apart from IRF3, other members of the IRF family of transcription factors also play important roles in MyD88-dependent signalling upon recognition of viral products through TLRs.

IRF7 has been described as a master regulator and is activated downstream of MyD88 in response to TLR7 and TLR9 ligation to induce IFN-I secretion [62]. In particular, plasmacytoid dendritic cells (pDCs) constitutively express IRF7 and respond swiftly by secreting IFN-I when exposed to viral products. The MyD88-IRF7 pathway is absolutely required for IFN-I secretion in pDCs [63]. MyD88 can directly associate with IRF7 which, when inactive, stays in the cytosol. IRF7 is subsequently phosphorylated and activated to form part of a complex composed of MyD88, IRAK1, IRAK4, TRAF3, TRAF6 and IKKα. The ubiquitin–ligase activity of TRAF6 is required for maximal activation of IRF7 [64]. The production of IFN-α in response to TLR9 ligands in pDCs requires activation of the phosphoinositide 3-OH kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway [63]. The intracellular phosphoprotein osteopontin (Opn-i) that has been described as essential for the development of T-helper 1 responses also plays a key role in MyD88-IRF7 pathway in pDCs stimulated with CpG and has been found as a component of the MyD88 signal transduction complex [65]. While MyD88, IRAK4, TRAF6 and IKKα are required for NFκB and IRF7 activation, IRAK1, TRAF3 and Opn-i selectively induce activation of IRF7 [66] (Fig. 2).

Like IRF7, IRF8 also interacts with MyD88 and mediates production of IFN-I and other inflammatory cytokines when activated by TLR9 engagement. IRF8 is a nuclear protein expressed in pDCs and also in conventional dendritic cells (cDCs) [67]. It has been implicated in TLR9-induced production of IFN-I and proinflammatory cytokines and also in the amplification phase of IFN-I production during viral infections [68].

IRF5 was essential for the MyD88-dependent production of IL-6 and IL-12 in TLR-mediated responses but was not required for IFN-α production [69].

IRF1 is induced by IFN-γ and also interacts with MyD88 upon TLR activation. MyD88-IRF1 interaction induces efficient translocation of IRF1 into the nucleus. The importance of IRF1 downstream of TLR engagement is supported by studies in IRF1-deficient cells showing impaired IFN-β secretion, inducible nitric oxide synthase (iNOS) activation and IL-12p35 production in response to TLR9 or TLR3 ligands [70].

TLR activation plays a key role in promotion of both humoral and the cell-mediated immunity. Optimal TLR signalling determines the combination of cytokines that will in turn define the outcome of the adaptive immune response. These receptors work in tandem with other receptors of the innate immune system to regulate innate responses, and they are key partners of NLRs, providing the first signal that is required for assembly of inflammasomes and further amplification of inflammation.

3 C-Type Lectin Receptors

The C-type lectin receptors or CLRs comprise another important family of PRRs that play a major role in antimicrobial immunity. The CLR superfamily is divided into 17 groups (I–XVII) according to their diverse structure and phylogeny including more than 1000 proteins [71, 72].

The CLRs were first described by the presence of a calcium-dependent carbohydrate-binding motif known as the carbohydrate recognition domain (CDR). However, it was later found that there were similar structurally conserved domains able to bind diverse ligands including glycans, lipids and proteins, among others [73]. These domains are now known as C-type lectin-like domains (CTLD) and are also characteristic of the CLRs [74]. Structurally CDRs and CTLD contain a motif composed of two loops harbouring conserved cysteine residues that stabilise the structure by establishing disulphide bridges between the two chains [73]. It is now clear as well that some CLRs can bind ligands independently from Ca^{2+} [74].

Given the vast and diverse number of proteins in the CLR superfamily, general characteristics of some membrane-bound CLRs and its signalling pathways are discussed below. Detailed information on particular receptors can be found in several comprehensive reviews [71, 73, 75].

3.1 Role of CLR in Microbial Recognition

CLRs which are mainly expressed in myeloid cells can be soluble or membrane bound and sense a wide variety of self and non-self ligands [75, 76]. The membrane-bound CLRs are classified into two groups: type-I CLRs that include receptors belonging to the mannose receptor family and group II CLRs that are part of the asialoglycoprotein receptor family. The latter includes the DC-associated C-type lectin 1 (dectin 1, also known as CLEC7A) subfamily and the DC immunoreceptor (DCIR or CLEC4A) subfamily [76]. CLRs appear more promiscuous than other PRRs and have been shown to bind several types of ligands. The CLRs expressed by DCs seem to preferentially recognise mannose, fucose and glucans, which allow them to recognise most types of pathogens including bacteria, fungi, viruses and parasites. Others, including Lox-1 or DNGR-1, respond to self ligands such as dead cells, while mincle or DC-SIGN can recognise ligands of microbial and self-origin and may mediate distinct responses to each one. CLRs have also been implicated in antitumor responses [72, 77] (Table 2).

The effects of CLRs upon ligand recognition are varied. Many CLRs can promote phagocytosis and endocytosis of the ligands, leading to degradation, which favours antigen presentation to T cells. Depending on the targeted CLR, the antigen will be directed towards either the MHC class I or MHC pathway or both [76]. Some CLRs can also promote microbicidal activity in innate cells, thereby enhancing pathogen clearance [71].

Table 2

Ligands and pathogen recognised by some CLRs

CLR	Ca^{2+} requirement	Ligands	Type of pathogen recognised
Dectin-2	Ca^{2+} dependent	α-mannans O-linked mannobiose-rich glycoprotein	*M. tuberculosis*, *S. mansoni*, *S. mansoni* egg antigen, *C. albicans*, *Malassezia* spp.
Mincle	Ca^{2+} dependent	α-mannose mannitol-linked glyceroglycolipid mannosyl fatty acids	*M. tuberculosis*, *C. albicans*, *Malassezia* spp.
DC-SIGN	Ca^{2+} dependent	High mannose surface layer A protein	HIV-1, Measles, Dengue, *Mycobacterium* spp., Influenza A, *S. mansoni* egg antigen, *Leishmania* spp., *H. pylori*, *Lactobacillus* spp., *M. leprae*, *Bacillus Calmette Guerin*
SIGNR3	Ca^{2+} dependent	High mannose and fucose	*L. infantum* *S. mansoni* egg antigen
DCIR	Ca^{2+} dependent	Not defined	HIV
Dectin-1	Ca^{2+} independent	β-glucans	*L. infantum* *C. albicans* *Mycobacterium* spp.
Mannose receptor (MR)	Ca^{2+} dependent	High mannose mannosylated lipoarabinomannan	*S. pneumoniae* *M. corti* *Mycobacterium* spp. *K. pneumoniae* *S. pneumoniae* *F. tularensis* *S. mansoni* egg antigen
DEC-205 (CD205)	Ca^{2+} dependent	Plasminogen activator	*Y. pestis*

3.1.1 CLRs in Antifungal Immunity

A number of CLRs contribute to antifungal responses including dectin 1, dectin-2, mincle and the mannose receptor (MR). However, to date only mutations in dectin-1 have been associated with increased susceptibility to fungal infections in humans [77] suggesting that several CLRs may have redundant roles in antifungal responses.

Dectin-1 recognises β-glucan moieties present in the cell wall of fungal pathogens including *Candida*, *Aspergillus*, *Pneumocystis* and *Coccidioides* species. Activation of dectin-1 involves receptor clustering and formation of the phagocytic synapse. This is a prerequisite for intracellular signalling. Activation of the dectin-1

pathway has been shown to be critical in inducing polarisation of Th1 and Th17 cells that are essential for fighting systemic and mucosal fungal infections, respectively [77]. Besides regulating differentiation of Th cells, engagement of the dectin-1 pathway has important effects on other cellular processes including phagocytosis, respiratory burst, autophagy and production of a number of proinflammatory mediators. Importantly, dectin-1 has been shown to operate together with the NLRP3 inflammasome as well as non-canonical caspase 8 inflammasomes to induce production of IL-1β [78–80]. Additionally, dectin-1 was required for IFN-I production in the context of *Candida albicans* infections through activation of the IRF5 pathway [81].

Dectin-2 has been also implicated in antifungal responses. It recognises α-mannans from *C. albicans* and O-linked mannobiose-rich residues from the *Malassezia* (formerly *Pityrosporum*) spp. [77]. Similar to dectin 1, dectin-2 promotes Th17 responses and also stimulates production of several cytokines including IL-23 and IL-1β in addition to reactive oxygen species (ROS).

3.1.2 CLRs in Bacterial Infections

Most of the evidence implicating CLRs in antibacterial immunity came from the study of CLRs in the context of mycobacterial diseases. A number of CLRs recognise PAMPs derived from mycobacteria including mincle, dectin 1, DC-SIGN (mice SIGNR3) and dectin-2 [72]. In vitro studies implicated multiple CLRs, namely, dectin-1, DC-SIGN and MR, in *Mycobacterium tuberculosis* infection control. However, when experiments were carried out in vivo, each of these CLRs appeared to be redundant [74]. Despite the apparent redundancy of these receptors during in vivo infection, the common signalling pathway involving CARD9 seems to be critical for protection since CARD9 deficiency in mice leads to uncontrolled bacterial replication and death [82].

CLRs have been also implicated in recognition of several other bacterial pathogens. DC-SIGN can recognise *Mycobacterium leprae, Bacillus Calmette–Guérin* (BCG), *Lactobacilli* spp. and *Helicobacter pylori* [76].

MR can also recognise bacteria such as *Streptococcus pneumoniae* [83], *Mycobacterium kansasii* [84] and *Francisella tularensis* [85]. However, the MR seems not to be essential during infection with these pathogens in vivo [76]. DEC-205 is another example of a CLR involved in recognition of bacterial pathogens. DEC-205 can bind to plasminogen activator expressed by *Yersinia pestis*. However, instead of eliciting a protective response against the pathogen, DEC-205 was found to promote dissemination of bacteria with detrimental consequences for the host [86], suggesting that pathogens can also take advantage of the internalisation pathway offered by these receptors to evade the immune response.

3.1.3 CLRs and Viruses

The interaction between CLRs and viruses is not always beneficial for the host. Similar to the case of DEC-205 and *Y. pestis*, CLRs can favour viral infections and transmission, with detrimental consequences for the host. In this regard, DC-SIGN interaction with HIV is one of the best characterised examples. Interaction of the viral protein gp120 with DC-SIGN favours viral entry into the cells, enhancing infection of $CD4^+$ T cells [87]. DC-SIGN has been also implicated in facilitating infection by influenza virus, which binds to the receptors through glycans on haemagglutinin [88]. Similar interactions between CLRs and other viruses such as dengue virus have been reported [76].

Despite the negative outcome that the interaction of some CLRs with viruses may have, in some cases it can be beneficial for the host and contribute to the antiviral response. CLEC9A is important for cross-presentation of antigens from vaccinia virus and *Herpes simplex* virus which is crucial for promoting cytotoxic antiviral responses [89, 90].

3.1.4 CLRs in Parasitic Invasion

CLRs have been implicated in recognition of carbohydrate moieties from parasites, particularly helminths. These parasites express a wide range of glycan moieties that can be recognised by different CLRs. DC-SIGN was implicated in the recognition of the soluble egg antigen of *Schistosoma mansoni* and other *Schistosome* spp. [91]. A range of other CLRs including MR, SIGNR1, SIGNR2 and dectin-2 have been implicated in recognition of *S. mansoni* antigens [74]. Dectin-2 has been shown to reduce Th2-mediated pathology in *S. mansoni* infection by promoting secretion of IL-1β through NLRP3 activation [92].

Finally, other infection models, particularly of central nervous system parasitic infections (e.g. neurocysticercosis), have shown that engagement of CLR by parasite ligands can contribute to pathology [74].

3.2 Signalling Downstream of CLRs

As previously mentioned, activation and signalling through CLRs has multiple outcomes including phagocytosis, activation of innate killing mechanisms by generation of microbicidal compounds such as ROS as well as production of inflammatory mediators. The immune response elicited by engagement of CLRs can be very different depending on the type of receptor, the cell-type expressing it and the nature of the ligand being recognised. Signalling pathways triggered by CLRs are only partially understood, and experimental evidence suggested that activation through CLRs such as MR, DEC-205 and cluster of differentiation (CD)-207 alone is insufficient to elicit gene transcription and/or microbicidal effector functions in myeloid cells, requiring cooperation of other receptors. The signalling pathways triggered by CLRs are complex and are often implicated in cross talk with other PRRs like TLRs

and NLRs. On the other hand, some other CLRs including dectin-1, dectin-2, SIGNR3 and mincle are self-sufficient and have been shown to directly couple PAMP recognition to myeloid cell activation and adaptive immunity [93].

According to the type of cytoplasmic signalling motifs and signalling potential, CLRs expressed in the myeloid linage can be classified into different categories: Syk-coupled CLRs, immunoreceptor tyrosine-based inhibitory motif (ITIM)-expressing CLRs, CLRs without immunoreceptor tyrosine-based activation motif (ITAM) or ITIM domains [71].

3.2.1 Syk-Coupled CLR

The self-sufficient CLRs rely on spleen tyrosine kinase (Syk) as an adaptor molecule. Syk binds to proteins with ITAMs. Phosphorylation of the tyrosine residues in the ITAM of the receptor by kinases of the Src family creates a dock for Syk, and a further conformational change activates Syk. Activation of Syk promotes its autophosphorylation and phosphorylation of other proteins downstream in the signalling cascade [93]. Some CLRs that use Syk require ITAM-bearing adaptors that associate with them in *trans* (e.g. FcRγ or DAP12); others can bind Syk directly through a single tyrosine-based motif in the intracellular domain. This domain has been named hemITAM [93].

Dectin-1 is the prototypical example for the Syk-coupled receptors with a hemITAM. It has been postulated that upon ligand recognition by dectin 1, dimerisation of the receptors occurs, bringing together two hemITAMs that serve as a docking site for Syk; dectin localises to specific lipid microdomains which are essential for signalling [71]. In myeloid cells dectin-1 uses the adaptor CARD9 to couple Syk signalling to NFκB activation. In humans, dectin-1 signalling triggers the formation of a protein complex that includes CARD9–Bcl10–MALT-1 which couples dectin-1 to the canonical NFκB pathway by activating NFκB subunit p65 and c-Rel. Dectin-1 also triggers the noncanonical NFκB RelB pathway [71, 94, 95]. MALT-1 has been shown to act as a pivotal regulator of the c-Rel subunit; silencing of MALT-1 specifically abrogated c-Rel activation in human DCs stimulated with the dectin-1 ligand curdlan but did not affect the other NFκB subunits. The proteolytic paracaspase activity of MALT-1 was required for c-Rel activation [96]. The ability of MALT-1 to activate c-Rel was linked to the production of IL-1β and IL-12p19 in cells stimulated with curdlan or in response to *C. albicans* infection and also induction of Th17 responses [96].

Signalling through dectin-1 not only promotes polarisation of Th cells into Th17 cells but also contributes in the development of the Th1 phenotype as well as cytotoxic $CD8^+$ cells [97–99].

Dectin 1–Syk-dependent activation of the NLRP3 inflammasome has also been reported in the context of fungal infection with *C. albicans*. Whereas pro-IL-1β synthesis is a

Syk–CARD9-dependent process, NLRP3 inflammasome activation requires ROS production and K^+ efflux. In agreement with these results, mice deficient in NLRP3 are more susceptible to *C. albicans* infection supporting a role for the inflammasome in antifungal responses [80].

As mentioned before, dectin-1 can also trigger the noncanonical activation of NFκB RelB subunit. This activity requires the kinase Raf-1. While Syk activates both canonical and noncanonical pathways, Raf-1 activation triggers acetylation of the NFκB p65 subunit which can modulate transcription in association with p50. Alternatively, acetylated p65 can bind the RelB activated by Syk to render it inactive. This results in negative regulation of the RelB-dependent cytokines that include IL-23p19, hence potentiating IL-12p70 formation, which in turn favours Th1-biased responses [95]. Additionally, dectin-1 signalling triggers activation of p38, ERK and JNK pathways as well as nuclear factor of activated T cells (NFAT), an inducible nuclear factor that binds the IL-2 promoter in activated T cells [71]. Activation of NFAT by dectin-1 agonists induces secretion of a particular set of cytokines in DCs combining proinflammatory cytokines together with high levels of IL-10 and IL-2 [71, 100]. Dectin-2 also signals through the Syk pathway, but since it lacks an intracellular signalling motif, dectin-2 associates with ITAM-containing FcRγ chains [101]. Although dectin-2 also triggers NFκB activation, it does it by selectively activating c-Rel through the recruitment of MALT-1, resulting in secretion of IL-1β and IL-23, important Th17 polarising cytokines. Dectin-2 has also been shown to trigger ERK, JNK and p38MAPK pathways in murine DCs [71].

3.2.2 ITIM-Expressing CLRs

Little is known about ITIM-bearing CLRs and the signalling pathways downstream of these receptors. Several human and mouse ITIM-expressing CLRs have been reported in immune cells including DCIR (DC-inhibitory receptor), MICL (myeloid inhibitory C-type lectin receptor), CLEC12B and Ly49Q [71, 93]. It has been proposed that activation of ITIM-bearing CLRs has a regulatory effect on myeloid cells, raising the threshold for cell activation.

DCIR has been shown to inhibit TLR signalling. Specifically, it has been shown that production of IFN-I upon stimulation of TLR9 or induction of IL-12 and TNF-α through TLR8 ligation is downregulated when DCIR is cross-linked with antibodies [93]. It has been proposed that activation of DCIR is followed by phosphorylation of the ITIM domain, leading to the recruitment of SHP-I and SHP-2, two phosphatases that inhibit TLR-dependent NFκB activation [71]. Similar findings have been reported for Ly49Q and other ITIM-expressing CLRs. However, signalling pathways have not yet been fully elucidated for these receptors, and the effect of their activation still needs to be addressed in vivo.

3.2.3 CLRs Without ITAM or ITIM Domains

Some CLRs engage signalling pathways that function independently of ITIM and ITAM domains. These signalling pathways seem to play a role in regulation and fine tuning of cells activated through other receptors rather than themselves acting as a trigger for cell activation. MR, DEC-205, DC-SIGN, SIGNR and langerin are some of the receptors that engage ITAM-/ITIM-independent signalling pathways [71].

DC-SIGN has been used as a model for ITAM-/ITIM-independent CLR signalling. DC-SIGN is involved in endocytosis of soluble ligands and particulates. Besides its role in endocytosis, DC-SIGN can trigger signalling cascades and act in coordination with other PRR like TLRs. It has been shown that DC-SIGN can modulate signalling triggered by TLR ligands. Particularly, binding of the *M. tuberculosis*-derived mannosylated lipoarabinomannan (ManLAM) to DC-SIGN impairs LPS-induced maturation of DCs and increases the production of the immunosuppressive cytokine IL-10. Although the nature of the ligand appears to regulate the outcome of DC-SIGN-mediated responses, it seems that the receptor has the ability to act as an immunomodulator. It has been shown that recognition of ManLAM, by DC-SIGN, leads to activation of a signalling complex that triggers the threonine–serine kinase Raf-1 which in turn mediates acetylation of the NFκB subunit p65. When TLR signalling is triggered, acetylation of p65 mediated by DC-SIGN-Raf-1 prolongs transcriptional activity of NFκB particularly enhancing IL-10 gene transcription and thus modulating TLR responses. IL-10 induction through DC-SIGN and TLR-dependent pathways was observed for several mycobacteria such as *M. tuberculosis*, *M. leprae* and *M. bovis,* BCG and also for *C. albicans* [102].

4 RIG-I-Like Receptors

The family of RIG-I-like receptors (RLRs) consists of a small group of cytosolic receptors that act as sensors of viral RNA. So far three members have been described: retinoic acid-inducible gene-I (RIG-I), its homolog the melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [103, 104]. Together with endosomal TLRs, the RIG-I-like receptors detect nucleic acids inside the cells, but in contrast to TLRs, which function in the lumen of endosomes, RLRs are located in the cytosol. Hence, RLRs sense pathogens that successfully bypassed detection in the extracellular or endosomal compartments and reached the cytosol. In contrast to TLRs that are mainly expressed in immune cells, RLRs are constitutively expressed in a wide variety of immune and nonimmune cells including epithelial cells of the central nervous system. Expression of RLRs is normally maintained at low levels in resting cells but is inducible by

viral infections, IFN-I stimulation and after TLR signalling in a IFN-I-independent fashion [105, 106].

4.1 Structure of RLRs

The three members of the RLR family show conserved structure and domain organisation, particularly in the case of RIG-I and MDA5. The receptors are organised in three different domains. The N-terminal region of MDA5 and RIG-I but not LGP2 contains a caspase recruitment and activation domains (CARD). Because LPG2 lacks CARD domains, it was considered an inhibitory receptor, but it was later found to play a positive role in MDA5 signalling. A central domain harbouring a DExD/H box RNA helicase that hydrolyses ATP and binds RNA is found in all three receptors. The C-terminal domain is involved in regulation and is partially responsible for ligand specificity [105, 107].

4.2 Ligands

RIG-I and MDA5 have been reported to recognise viruses from different families including Paramyxoviridae, Flaviviridae, Rhabdiviridae and Picornaviridae. RIG-I has been implicated in recognition of hepatitis C virus (HCV), Sendai and Newcastle viruses and vesicular stomatitis virus. MDA5 has been shown to be involved in recognition of poliovirus and dengue virus which is also recognised by RIG-I [107]. While LGP2 has the ability to bind RNA, its role during viral infections it is not yet clear. RIG-I was initially described as a sensor for double-stranded RNA (dsRNA), including the synthetic ligand poly(I:C) [104]. It is now clear that RIG-I recognises RNA sequences harbouring a triphosphorylated 5′ end (5′ppp). The 5′ppp end serves as a label for non-self RNA [108]. Although the length of the RNA sequence is not an absolute determinant, RIG-I has greater affinity for short RNA molecules with the 5′ppp end and dsRNA motifs [109]. 5′-hydroxyl (5′-OH) and 3′-monophosphoryl short RNA molecules with double-stranded stems generated by RNase L have been also reported to activate RIG-I, suggesting that RIG-I could recognise ligands derived from viral genomes, viral replication intermediates, viral transcripts or RNA cleaved by RNase L during infections [107]. Interestingly, RIG-I was also implicated in the sensing of dsDNA, specifically B-forms of poly(dA:dT). Sensing of poly(dA:dT) required the DNA-dependent RNA polymerase III that is able to synthesise 5′ppp RNA from poly(dA:dT) [110].

Ligands for MDA5 are less well characterised. It is known that MDA5 can be activated by poly(I:C) which suggests that it acts as a sensor for dsRNA [111]. Until now there are no reported ligands for LGP2.

4.3 Signalling Pathways Triggered by RLRs

In the absence of its ligand, RIG-I adopts an autorepressed form, preventing the CARD domains from signalling by blocking dsRNA binding to the helicase or modification of the CARD domains by ubiquitination enzymes. Binding of the RNA induces a

conformational change that together with ATP hydrolysis results in release of the CARD domains, leaving them available for signalling interactions. CARDs are polyubiquitinated. This modification triggers formation of a complex formed by four RIG-I molecules [105, 107]. MDA5 has been reported to form a polar filamentous oligomer around the dsRNA ligand, which is regulated by ATP hydrolysis [112].

Signalling downstream of the complex formed by RIG-I relies on the mitochondrial protein MAVS (mitochondrial antiviral signalling), a protein that also functions as an adaptor used by the NLR member NOD2 in the context of viral infections. MAVS is anchored to the mitochondrial outer membrane and harbours an N-terminal CARD domain that allows it to establish homotypic interactions with the CARD domains of RIG-I and also MDA5 [113, 114]. During viral infections, MAVS aggregates and localises to the outer mitochondrial membrane. Together with maintenance of the mitochondrial membrane potential, these events are critical for induction of IRF3 translocation and production of IFN-I [115].

Signalling downstream of MAVS involves NEMO, IKK and TBK1. Ubiquitination is critical for downstream signalling, and it is sensed by NEMO through its ubiquitin-binding domains, allowing recruitment of IKK and TBK1 which in turn phosphorylate IκBα and IRF3, respectively, promoting translocation of IRF3 and the NFκB subunits p50 and p65 [107]. Different members of the E3 ligases, namely, TRAF6, TRAF2 and TRAF5, have been reported to be recruited to MAVS complexes and participate in the antiviral responses elicited by RIG-I engagement [107].

Besides promoting innate responses, RLRs play an important role in modulating cell-mediated immunity. IFN-I secretion promotes maturation of APCs and expression of MHC class I molecules in most cell types and is required to promote T-cell survival and expansion. It has been also proposed that interferons can promote the cytolytic activity of cytotoxic T lymphocytes and natural killer cells, enhancing the antiviral response [116, 117].

In conclusion RLRs are important PRRs in the context of viral infections. RLR activation seems to be crucial for the onset of antiviral responses and also serves to upregulate expression of other PRRs including TLRs. Enhancement of TLR expression by RLR has been shown to have an impact on the MyD88 signalling pathway and to play an important role in some viral infections. Cross talk between RLRs and other PRRs can also enhance inflammation, having negative consequences for the host [105]. Coordinated cross-regulation of the different signalling pathways is not only important for infection control but also for preventing exacerbation of inflammation and damage to the host.

5 NOD-Like Receptor Family

The members of the nucleotide-binding oligomerisation domain (NOD)-like (NLR) family have emerged as pivotal sensors of infection and stress in intracellular compartments, capable of orchestrating innate immunity and inflammation in response to harmful signals within the cell. NLRs detect a wide range of signals including the presence of intracellular PAMPs that function as flags for cellular invasion; other NLRs are activated following loss of cell membrane integrity, ion imbalance, radical oxygen species (ROS) or sensing of extracellular ATP [118].

NLRs have the ability to activate NFκB signalling; some of them function as scaffolds for the formation of a multiprotein complex known as inflammasomes required for the generation of bioactive IL-1β and IL-18 and can also trigger cell death by a mechanism known as pyroptosis [118].

Although the primary physiological role of NLRs is related to host defence against infection, in the last decade, it became increasingly clear that NLRs play a vital role in homeostasis as illustrated by many inflammatory and noninflammatory diseases that are linked to dysregulated NLR signalling [119].

Vertebrate NLRs have been the subject of intense research, while knowledge on invertebrates is limited, probably in part due to the absence of NLRs in invertebrate model organisms like *D. melanogaster* and *Caenorhabditis elegans* [120, 121]. In any case, studies revealing that NLRs are conserved across different species and kingdoms suggest they are an essential product of evolution. This is consistent with their conservation from sponges to humans and the finding that plants also express NB-LRR receptors with remarkable structural and functional similarities, although the relation of animal NLRs and the latter seems to be a result of convergent evolution rather than shared ancestry [121]. In the following section structure, the ligands and function of NLRs are discussed, with a particular focus on non-inflammasome-related NLRs, whereas inflammasome-forming NLRs are introduced in the following chapters.

5.1 Structure and Triggers of NLRs

NLRs are cytosolic sensors for microbes, endogenous danger signals and exogenous insults. The defining feature of NLR family members is the presence of a nucleotide-binding domain, the NACHT domain (acronym standing for NAIP (neuronal apoptosis inhibitor protein), CIITA (class II transcription activator), HET-E and TP-1 (telomerase-associated protein)) and a second domain harbouring a leucine-rich repeat [122]. Based on in silico studies, 22 human and 34 murine NLRs have been identified so far [123]. All the NLRs share common structural features and are organised

in three functional domains including the aforementioned central NACHT (or NBD) domain necessary for oligomerisation, the C-terminal LRR that confers ligand recognition specificity and an N-terminal protein–protein interaction domain required for signal transduction [123].

The NLRs are subdivided into four subfamilies according to the type of N-terminal effector domain: NLRA, NLRB, NLRC and NLRP. The NLRA subfamily contains only one member named CIITA which presents an acidic transactivation domain [122] involved in transcriptional regulation of the MHC class II genes [123]. The other three subfamilies are characterised by the presence of homotypic protein–protein interaction modules that are involved in recruitment of signal transduction molecules. The NLRB subfamily is distinguished by the presence of the baculovirus inhibitor repeat (BIR) domain. The presence of a CARD is a feature of the NLRC subfamily, while members of the NLRP family contain a pyrin domain (PYD). Finally NLRX1, a CARD-related X effector domain of unknown function shows no strong homology to the N-terminal domain of any other NLR subfamily member [122]. Members of each subfamily are listed in Table 3.

NLRs detect a wide range of ligands of diverse origins. As in the case of TLRs, NLRs can detect PAMPs, particularly when these PAMPs reach the cytosol. Different bacterial components such as bacteria muramyl dipeptide (MDP) [124, 125], flagellin [126] and bacterial secretion systems [127, 128] function as flags for cellular invasion and trigger NLR activation and signalling. NLRs can also be activated by stress or danger signals including loss of cell membrane integrity as induced by certain bacterial toxins that form pores in the cell membrane [122, 129, 130]. Endogenous signals of damage also act as triggers of NLRs, such as membrane rupture caused by insoluble crystals [131], extracellular ATP [132–134], ion imbalance [135–137] and reactive oxygen species (ROS) [118]. Sensing the loss of cellular integrity allows NLRs to act as a backup system if a pathogen has bypassed detection in the extracellular space and also ensures recognition of DAMPs. Accordingly, the cellular inflammatory programme following NLR triggering is complex and varies between NLR members.

The NLRs have been shown to function as scaffolds for inflammasome formation. These are high molecular weight oligomeric complexes that act as caspase 1-activating platforms in response to microbial components or sterile danger and stress signals. Inflammasome complexes are formed by a sensor molecule, often but not exclusively, a member of the NLR family which connects to caspase 1 via an adaptor protein named ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) equipped with two death-fold domains (pyrin domain and caspase activation and recruitment domain (CARD)). The adaptor ASC interacts with the inflammasome sensor molecules via the pyrin

Table 3
NOD-like receptors nomenclature

NLR member	Family	Domain structure
CTIIA	NLRA	(CARD)-AD-NACHT-NADLRR
NAIP	NLRB	(CARD)-AD-NACHT-NADLRR
NOD1	NLRC	(CARD)-AD-NACHT-NADLRR
NOD2	NLRC	CARD2x-NACHT-NAD-LRR
NLRC3	NLRC	CARD-NACHT-NAD-LR
NLRC4	NLRC	CARD-NACHT-NAD-LR
NLRC5	NLRC	CARD-NACHT-NAD-LR
NLRP1	NLRP	PYD-NACHT-NAD-LRR-FIIND-CARD
NLRP2	NLRP	PYD-NACHT-NAD-LRR
NLRP3	NLRP	PYD-NACHT-NAD-LRR
NLRP4	NLRP	PYD-NACHT-NAD-LRR
NLRP5	NLRP	PYD-NACHT-NAD-LRR
NLRP6	NLRP	PYD-NACHT-NAD-LRR
NLRP7	NLRP	PYD-NACHT-NAD-LRR
NLRP8	NLRP	PYD-NACHT-NAD-LRR
NLRP9	NLRP	PYD-NACHT-NAD-LRR
NLRP10	NLRP	PYD-NACHT-NAD-LRR
NLRP11	NLRP	PYD-NACHT-NAD-LRR
NLRP12	NLRP	PYD-NACHT-NAD-LRR
NLRP13	NLRP	PYD-NACHT-NAD-LRR
NLRP14	NLRP	PYD-NACHT-NAD-LRR
NLRX1	NLX	X-NACHT-NAD-LRR

AD acidic activation domain, *CARD* caspase activating and recruitment domain, *LRR* leucine-rich repeat, *NACHT* NAIP (neuronal apoptosis inhibitor protein), C2TA (MHC class 2 transcription activator), HET-E (incompatibility locus protein from Podospora anserina) and TP1 (telomerase-associated protein), *PYD* pyrin domain, *NAD* NACHT-associated domain

domain and triggers the assembly of a large protein speck consisting mainly of multimers of ASC dimers. The CARD domains recruit caspase 1 to induce self-cleavage and activation, which in turn will allow processing of pro-IL-1β and pro-IL-18 into the active inflammatory forms and their release via a nonclassical secretion pathway [138].

The increasing number of studies on NLRs in the context of inflammasome formation in the last decade illustrates the

important and varied roles of these complexes in immunology, linking inflammasome formation not only to antimicrobial responses but also autoimmunity. The following chapters will discuss in detail the role of NLRs as part of inflammasomes. However, several members of the mammalian NLR family exert important roles in immunity beyond inflammasome signalling. Here we highlight the emerging roles of several members of the non-inflammasome NLRs, CIITA, NOD1, NOD2, NLRC3, NLRC5 and NLRX1.

5.1.1 CIITA

CIITA plays a critical role in immune responses, acting as a transcriptional coactivator that regulates major histocompatibility complex (MHC) class I and II genes. Its importance as a regulator of the MHC genes was identified after finding that patients with an autoimmune condition known as bare lymphocyte syndrome have a 24 amino acid deletion splice mutant of CIITA [139]. Depending on the cell type, CIITA can be constitutively expressed (e.g. in DCs, macrophages and other cells with high MHC II expression) or can be induced by IFN-γ in a wide range of cell types [140].

In addition to the conventional tripartite architecture of NLRs, CIITA harbours three additional N-terminal domains including an acidic domain (AD), a guanosine-binding domain (GB-domain) and a Pro-Ser-Thr domain (PST domain). Although CIITA does not bind DNA directly, the AD and GBD domains mediate interactions with transcription factors, DNA-binding transactivators and chromatin-remodelling enzymes forming a complex known as the enhanceosome [123].

Although NLRs are mostly cytoplasmic receptors, CIITA can also reside in the nucleus [119]. A nuclear localisation signal is present in the GB-domain that allows trafficking into the nucleus [123].

CIITA function is regulated by phosphorylation. Several protein kinases (PK) such as PKA, PKC, glycogen synthase kinase 3 and casein kinase 2 can phosphorylate CIITA on different sites affecting its activity. CIITA possesses acetyltransferase (AT) and kinase activities, both of which are needed for effective transcription of MHC class I and II genes [123]. Although CIITA has been primarily characterized as a transcriptional regulator of MHC genes, it also regulates transcription of over 60 immunologically important genes, including IL-4, IL-10 and several thyroid-specific genes [140, 141].

Given the distinctive immunomodulatory role of CIITA, the potential of other NLR members to exert comparable effects is being addressed. It was recently proposed that NLRC5 can act as a class I transactivator. NLRC5 presents a similar domain structure to CIITA, and it has been proposed to assemble a multiprotein complex similar to the enhanceosome on MHC class I promoters [142].

5.1.2 NOD1 and NOD2

NOD1 and NOD2 were the first members of the NLR family to be described. Also known as CARD4 and CARD15, NOD1 and NOD2 were first described as receptors for LPS. However, it was

later confirmed that the LPS preparations used for the experiments were contaminated by peptidoglycan moieties [119].

Gram-positive and Gram-negative bacteria synthesise peptidoglycan although they may present different motifs. Both Gram-positive and Gram-negative bacteria express muramyl dipeptide (MDP) (MurNAc-L-Ala-D-γ-Gln), but only Gram-negative bacteria and a limited number of Gram-positive bacteria express iEDAP (γ-D-glutamyl-meso-diaminopimelic acid); when it also includes the D-alanine residue from the peptidoglycan, the ligand is termed TriDAP. The difference between MDP and iEDAP is the replacement of the meso-diaminopimelic acid in the iEDAP by an L-lysine residue in MDP (Fig. 3a). NOD1 recognises the iEDAP/TriDAP, while NOD2 recognises MDP [124, 143, 144]. More recently, N-glycolyl MDP was shown to be a more potent activator of NOD2 [145]. The fact that iEDAP is mainly expressed in Gram-negative bacteria led to the idea that NOD1 was acting as a sensor for this type of microorganism. Indeed, NOD1 was shown to be involved in recognition of many different Gram-negative bacteria including *Helicobacter pylori* [146], *Pseudomonas aeruginosa* [147] and *Shigella flexneri* [148]. However, NOD1 has also been implicated in defence against Gram-positive bacteria including *Listeria monocytogenes* [149] and *Streptococcus pneumoniae* in a model of coinfection with Gram-negative bacteria [150, 151] and more surprisingly against the parasite *Trypanosoma cruzi*, etiological agent of Chagas disease [152].

NOD2 is seen as a more general sensor because its ligand MDP is widely expressed. Experimental evidence has confirmed a role for NOD2 during infection with *M. tuberculosis* [145], *Listeria monocytogenes* [153] and *Toxoplasma gondii* [154]. NOD2 may also play a role in antiviral immunity; NOD2-deficient mice exhibited a marked susceptibility to respiratory syncytial virus infection compared to the wild-type counterparts [155].

NOD2 has been also linked to pathology in Crohn's disease, an inflammatory disease that mainly affects the ileum and colon. An increased susceptibility to developing this condition was linked to several mutations in NOD2, although the aetiology is not fully understood. It has also been postulated that NOD2 could negatively regulates TLR-mediated inflammation since NOD2 deficiency or a mutation related to Crohn's disease increased Toll-like receptor 2-mediated activation of NFκB and Th1 responses. Moreover, NOD2 inhibited TLR2-driven activation of NFκB [156].

NOD1 and NOD2 expression has been reported in a wide variety of cells including dendritic cells [157], monocytes/macrophages [158], keratinocytes [159], lung and intestinal epithelial cells [160, 161] and endothelial cells [162]. Although several cell types constitutively express NOD1 and NOD2, its expression can also be induced in response to cytokines [163], TLR ligands and bacteria [164, 165]. Signalling through NOD1 and NOD2

ultimately triggers NFκB activation and MAPK. The IRF pathway and IFN-I transcription can be also triggered by NOD receptors (Fig. 3b).

The first step in the signalling cascade involves dimerisation of the receptors [119]. Although both are considered cytosolic receptors, association with the plasma membrane seems to occur after ligand binding [166].

It has been proposed that bacterial peptidoglycans are internalised in endosomes and can access the cytosol by exiting the vesicles through channels including hPepT1 and SLC15A. Scavenger receptors such as MARCO and SR-A have been implicated in rapid internalisation of NOD ligands [123]. After recognition of the ligand and assembly of the dimers, a protein adaptor known as RICK/RIP2 is an ubiquitinated interaction with the CARD domains of NOD1 or NOD2 [167]. RICK participates in the recruitment of TAK1; it also promotes ubiquitination of IκKγ which acts as a regulator of the IκK complex. NEMO, another regulator of NFκB, is also recruited and facilitates TAK1 recruitment to the complex. Formation of this complex promotes phosphorylation and targeting of the IκK complex subunits for proteasomal degradation, promoting release of NFκB [119, 123]. Activation of NOD1 and NOD2 also triggers activation of the MAPK pathway although this has received less attention.

Finally, NOD receptors have been shown to activate the IRF pathway (Fig. 3b). NOD2 induces type-I IFN secretion upon recognition of viral single-stranded RNA or during infections with respiratory syncytial virus and influenza virus [155]. Induction of IFN-I involves activation of a RICK-independent pathway with formation of a complex with the protein MAVS, the adaptor used by RLRs during viral infections [118].

NOD1 has been shown to induce IFN-I production after recognition of iE-DAP. Binding of iE-DAP to NOD1 triggered RICK signalling and also the recruitment of TRAF3 which was shown to trigger TBK1, IKKε and activation of IRF7, inducing IFN-β production. This ultimately led to activation of the transcription factor complex ISGF3 and secretion of CXCL-10 and further production of IFN-I [168].

As seen for TLRs, activation of the NFκB pathway through NOD1 and NOD2 receptors promotes expression of several

Fig. 3 (continued) of the IκK complex. NEMO, another regulator of NFκB, is also recruited and facilitates TAK1 recruitment to the complex. Formation of this complex promotes phosphorylation, and targeting of the IκK complex subunits for proteasomal degradation promoting release of NFκB of NOD1 and NOD2 also triggers activation of the MAPK pathway. The type-I IFN pathway can also be activated by NOD receptors. NOD2 induces type-I IFN secretion after recognising viral single-stranded RNA or during viral infections with respiratory syncytial virus and influenza virus. A RICK-independent pathway is triggered for IFN-I production. This pathway involves formation of a complex with the protein MAVS. Translocation of IRF3 and IRF7 takes place

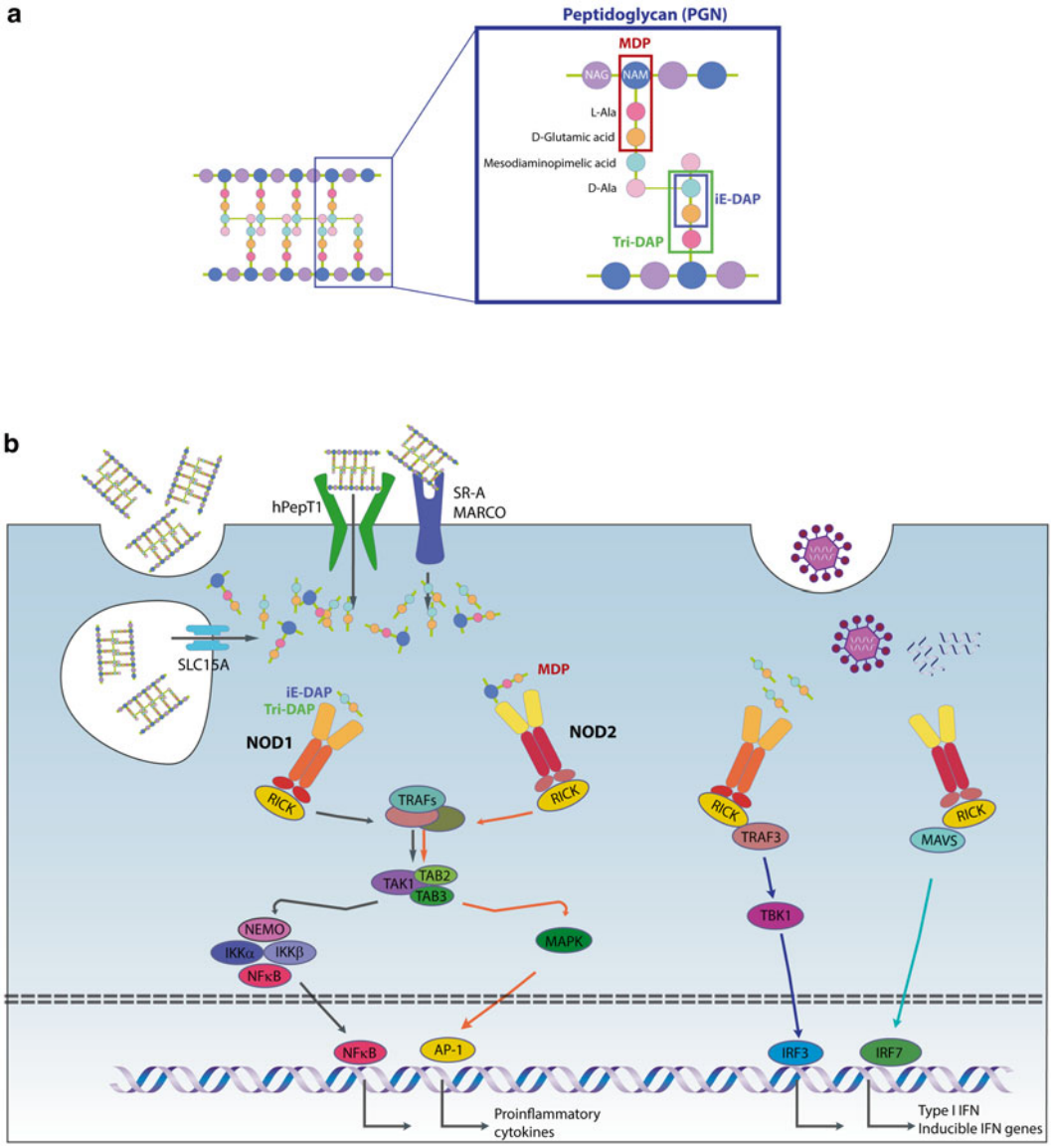

Fig. 3 Ligands and signalling pathways for NOD1 and NOD2. (**a**) *Structure of peptidoglycan (PGN) of Gram-negative bacteria.* PGN is composed of the alternating amino sugars N-acetylglucosamine (NAG) and N-acetyl muramic acid (NAM) cross-linked by ß1-4 linkages. NAG and NAM units are cross-linked by stem peptides containing amino acids such as D-glutamic acid and D- or L-alanine. Generally, the third position amino acid in Gram-positive bacteria is L-lysine (Lys), while in Gram-negative bacteria it is meso-2,6-diaminopimelic (meso-DAP) acid. Gram-positive bacteria have peptide stems usually cross-linked through an interpeptide bridge (normally glycine), whereas Gram-negative bacteria peptide stems are usually directly cross-linked. Abbreviations: *iE-DAP* D-g-glutamyl-meso-DAP, *Tri-Dap* L-Ala-γ-D-Glu-mDAP, *MDP* muramyl dipeptide. (**b**) *Signalling pathways for NOD1 and NOD2.* Transport of bacterial PNG fragments is mediated by endosomal SLC15A channel, the hPepT1 plasma membrane transporter or scavenger receptors (SR-A, MARCO). MDP and iE-DAP from PNG activates NOD1 and NOD2, respectively. Direct or indirect sensing of PNG leads to NOD1 and NOD2 dimerisation and relocalisation to the plasma membrane and triggers and recruitment of the adaptor RICK. RICK participates in recruitment of TAK1; it also promotes ubiquitination of IκKγ which acts as a regulator

inflammatory factors including inducible nitric oxide synthase, cyclooxygenase 2, adhesion molecules and proinflammatory cytokines and chemokines such as such as TNF-α, IL-8, IL-6 [118, 119]. Relatively little is known about how NOD1 and NOD2 signalling pathways are regulated. It has been suggested that the ubiquitin-editing enzyme A20 plays a key role in regulating RICK activity upon MDP recognition by NOD2. A20 deficiency amplified the responses to MDP in cells by increasing RICK ubiquitination, resulting in prolonged NFκB signalling and increased production of proinflammatory cytokines. A similar phenotype has been found in A20-deficient mice [169]. Caspase 12 has been also implicated in negative regulation of the NOD signalling pathway by binding to RIPK2 and destabilising the complex between RICK and TRAF6, ultimately inhibiting the ubiquitin-ligase activity of the complex [170].

NOD agonists have also been implicated in enhancing antigen-specific antibodies and T-cell responses when combined with TLR ligands. One example of the importance of NOD receptor in enhancing adaptive responses is given by the diminished responses observed in NOD1-deficient mice immunised with a model protein antigen (ovalbumin) formulated in complete Freund's adjuvant (CFA). CFA contains mycobacterial cell wall elements known to activate both NOD and TLR receptors. NOD1-deficient mice showed lower frequencies of antigen-specific IFN-γ, IL-17 and IL-4-producing $CD4^+$ and $CD8^+$ T cells, and diminished antibody titres compared to wild-type mice. Besides, increased susceptibility of NOD1-deficient mice to *Helicobacter pylori* infection was linked to diminished urease-specific IgG2c titres compared to wild-type mice, whereas IgG1 titres remained the same. This suggested that NOD1 deficiency was linked to a diminished type 1 immune response [171]. This is in agreement with other studies showing that NOD1 and NOD2 agonists in combination with TLR3, TLR4 and TLR9 agonists synergistically induce IL-12 and IFN-γ production in DCs to induce Th1-biased immune responses [157]. Another example of the potential synergistic effects of TLR-NOD on adaptive responses was given by a study showing that a chimeric NOD2/TLR2 agonist not only induced dendritic cell maturation and proinflammatory cytokine secretion in vitro but also boosted systemic and mucosal immune responses after parenteral immunisation of mice when formulated as a nanoparticle-based vaccine carrying the HIV antigen Gag p24 [172].

5.1.3 NLRC3

NLRC3 is another member of the NLR family, but instead of promoting inflammation, NLRC3 acts as a negative regulator of several innate receptors. It was first described as a negative regulator of T-cell proliferation, an effect mediated by downregulation of the transcription factors NFκB, AP-1 and NFAT. NLRC3 expression was shown to be high in T cells and downregulated upon activation [173].

The same receptor was also shown to have regulatory effects on TLR-mediated signalling by directly interacting with TRAF6 as evidenced by the increased proinflammatory cytokine production in NLRC3-deficient mice upon stimulation with LPS [174].

Lastly, NLRC3 has been shown to act as a negative regulator of the cytosolic DNA sensor STING by directly associating with it and TBK1, preventing the interaction between the two of them reducing production of IFN-I in response to cyclic diguanylate monophosphate (c-di-GMP) and DNA viruses [175]. NLRC3 illustrates how PRRs can regulate each other to fine-tune the immune response in the host.

5.1.4 NLRC5

NLRC5 is structurally similar to CIITA and is also involved in regulation of MHC genes, more specifically as a class I transactivator. It has been described as the largest member of the NLR family, harbouring an atypically long LRR motif and death domain [142]. Several isoforms of NLRC5 with unknown function have also been described [176]. Expression of NLRC5 has been reported in several cell types, mainly of haematopoietic origin. It is highly expressed in lymphoid tissues like the spleen and lymph nodes and has also been reported to be expressed in the bone marrow. NLRC5 has been documented in T cells, B cells and mononuclear cells [176].

NLRC5 promotes the expression of conventional MHC class I genes (HLA-A, HLA-B, HLA-C) as well as nonconventional MHC class I genes and proteins like HLA-E. NLRC5 regulation was shown to be exclusive for MHC class I genes and not MHC class II, whereas as previously mentioned, CTIIA is a MHC-II regulator [177].

Besides its function as a class I transactivator, NLRC5 has been implicated in negative regulation of NFκB and IRF pathways. NLRC5 can inhibit the IKK complex and RIG-I/MDA5 function. By blocking phosphorylation of IKKα and IKKβ, NLRC5 blocked the activation of NFκB. Additionally, interaction with RIG-I and MDA5 was shown to inhibit IFN-I responses triggered by RIG-like receptors [178]. Despite this, macrophages and dendritic cells derived from NLRC5-deficient mice did not exhibit compromised production of IFN-β, IL-6 or TNF-α when stimulated with RNA viruses, DNA viruses or bacteria [179]. Hence, the regulation could be cell-type specific.

5.1.5 NLRX1

This member of the NLR family has been one of the most controversial regarding its function. Although NLRX1 was first described as a negative regulator of antiviral responses, particularly by down-regulating IFN-I secretion in response to viral infections [180], other studies could not confirm this effect. Additionally, knockout mice showed normal IFN-β production after influenza A infection or systemically administered poly (I:C) [181, 182]. In other set of studies NLRX1 was shown to negatively regulate TLR-mediated activation of NFκB and JNK signalling pathways [183].

6 Newly Described PRRs

The recent discovery of NLRs and RLRs opened new avenues for studying other cytosolic receptors, leading to discovery of new PRRs. In the past decade, several proteins have been described as intracellular sensors for nucleic acids, in particular the family of AIM2-like receptors or ALRs together with the DNA sensor cGAS.

6.1 AIM2-Like Receptors

The family of AIM2-like receptors comprises four members in humans and six in mice. The founding member of this new family of PRRs is the absent in melanoma 2 (AIM2) protein that was described in 2009 [184–186]. The members of the ALR family are characterised by the presence of a HIN200 domain, also known as IFI200. Three members of the family in humans (MNDA, PYHIN1 and AIM2) present an N-terminal PYD domain, whereas the fourth member IFI16 harbours two tandem HIN200 domains and one PYD. The HIN200 domains can interact with cytosolic dsDNA, either of viral or bacterial origin, triggering IFN-I production [187]. Also, the PYD domain can recruit the adaptor ASC to form an inflammasome and lead to production of bioactive IL-1β. Importantly, until now only AIM2 and IFI16 have been reported as PRRs. The fact that ALRs have only been described in mammals suggests they are a novel family of receptors that appeared later in evolution [187].

6.2 cGAS and STING

cGAS has recently emerged as a major sensor for cytosolic DNA and together with the adaptor protein STING which is widely expressed in various cell types, they contribute to DNA sensing from different origins including DNA from viruses, self DNA and sensing of bacterial cyclic dinucleotides such as c-di-GMP and c-di-AMP [107, 188, 189]. Activation of STING triggers trafficking of the adaptor from the endoplasmic reticulum to the Golgi apparatus for the assembly of protein complexes with TBK1 [190]. The STING pathway can trigger IFN-I production and has been also reported to recruit STAT6, promoting secretion of chemokines including CCL-2, CCL-20 and CCL-26 [191].

The cyclic GMP-AMP synthase (cGAS), a member of the nucleotidyltransferase family, has been identified as a cytosolic DNA sensor that contributes to the production of IFN-I. cGAS catalyses the synthesis of cyclic GMP-AMP (cGAMP) from ATP and GTP, which in turn acts as a second messenger for STING [192, 193].

Deficiency of cGAS in vivo abolished type-I IFN production in response to cytosolic DNA [194]. cGAS signalling has proven important in the context of several viral infections caused by DNA viruses and interestingly also contributes to antiviral immunity to RNA viruses [195, 196].

7 Conclusion

Overall, the discovery of PRRs has changed the way innate immunity is viewed and has brought it back to the foreground. Unravelling the molecular mechanisms of immune recognition revealed the interconnection between innate and adaptive immunity, supporting the notion that innate recognition is a key event that allows the host to mount the most effective immune response.

Even though recognition of microbes or abnormal-self is restricted to a finite number of molecular patterns, these confer a significant degree of specificity allowing for tailored responses. During infection several receptors are simultaneously activated, triggering specific combinations of signalling pathways that converge on NFκB, MAPK and IRFs. These pathways that share common players downstream from the PRRs engage in cross-talk that can result in synergy, enhancement, negative regulation and fine-tuning of gene expression. Moreover, given that the different PRRs display differential expression patterns that can be tissue or cell-type specific, those interactions will be dependent on the context in which the activation takes place. PRRs have been shown to cross-regulate each other, and defining how this cross-talk is regulated is crucial to better understand how pathways that seem to converge on the same master regulators can facilitate different outcomes. This will certainly open new avenues for treatment of inflammatory diseases and immune deficiencies and will make it possible to exploit PRRs as a means to induce protective immunity through vaccination.

References

1. Cooper EL (2010) Evolution of immune systems from self/not self to danger to artificial immune systems (AIS). Phys Life Rev 7(1):55–78. doi:10.1016/j.plrev.2009.12.001
2. Metschnikoff E (1884) Ueber eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampf der Phagocyten gegen Krankheitserreger. Archiv f Pathol Anat 96(2):177–195. doi:10.1007/BF02361555
3. Wilson JDKE (2007) Sir Frank Macfarlane Burnet 1899–1985. Nat Immunol 8(10):1009. doi:10.1038/ni1007-1009
4. Landsteiner K (1933) Die Spezifität des serologischen Reaktionen. Springer, Berlin
5. Burnet FM (1959) The clonal selection theory of acquired immunity. Vanderbilt University Press, Nashville
6. Janeway CAJ (1989) Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54:1–13
7. Liu Y, Janeway CAJ (1991) Microbial induction of co-stimulatory activity for CD4 T-cell growth. Int Immunol 3(4):323–332
8. Liu Y, Janeway CAJ (1992) Cells that present both specific ligand and costimulatory activity are the most efficient inducers of clonal expansion of normal CD4 T cells. Proc Natl Acad Sci U S A 89(9):3845–3849
9. Matzinger P (1994) Tolerance, danger, and the extended family. Annu Rev Immunol 12:991–1045. doi:10.1146/annurev.iy.12.040194.005015
10. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86(6):973–983
11. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the Drosophila Toll protein signals activation of

adaptive immunity. Nature 388(6640):394–397. doi:10.1038/41131

12. Schaefer L (2014) Complexity of danger: the diverse nature of damage-associated molecular patterns. J Biol Chem 289(51):35237–35245. doi:10.1074/jbc.R114.619304
13. Broz P, Monack DM (2013) Newly described pattern recognition receptors team up against intracellular pathogens. Nat Rev Immunol 13(8):551–565. doi:10.1038/nri3479
14. Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. Science 327(5963):291–295. doi:10.1126/science.1183021
15. Hashimoto C, Hudson KL, Anderson KV (1988) The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell 52(2):269–279
16. Gay NJ, Keith FJ (1991) Drosophila Toll and IL-1 receptor. Nature 351(6325):355–356. doi:10.1038/351355b0
17. Dinarello CA (1991) Interleukin-1 and interleukin-1 antagonism. Blood 77(8):1627–1652
18. Heguy A, Baldari CT, Macchia G, Telford JL, Melli M (1992) Amino acids conserved in interleukin-1 receptors (IL-1Rs) and the Drosophila Toll protein are essential for IL-1R signal transduction. J Biol Chem 267(4):2605–2609
19. Sen R, Baltimore D (1986) Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. Cell 47(6):921–928
20. Kawai T, Akira S (2009) The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol 21(4):317–337. doi:10.1093/intimm/dxp017
21. Buchmann K (2014) Evolution of innate immunity: clues from invertebrates via fish to mammals. Front Immunol 5:459. doi:10.3389/fimmu.2014.00459
22. Botos I, Segal DM, Davies DR (2011) The structural biology of Toll-like receptors. Structure 19(4):447–459. doi:10.1016/j.str.2011.02.004
23. Lee BL, Moon JE, Shu JH, Yuan L, Newman ZR, Schekman R, Barton GM (2013) UNC93B1 mediates differential trafficking of endosomal TLRs. Elife 2,e00291. doi:10.7554/eLife.00291
24. Pifer R, Benson A, Sturge CR, Yarovinsky F (2011) UNC93B1 is essential for TLR11 activation and IL-12-dependent host resistance to *Toxoplasma gondii*. J Biol Chem 286(5):3307–3314. doi:10.1074/jbc.M110.171025
25. Jin MS, Lee JO (2008) Structures of the Toll-like receptor family and its ligand complexes. Immunity 29(2):182–191. doi:10.1016/j.immuni.2008.07.007
26. Omueti KO, Beyer JM, Johnson CM, Lyle EA, Tapping RI (2005) Domain exchange between human Toll-like receptors 1 and 6 reveals a region required for lipopeptide discrimination. J Biol Chem 280(44):36616–36625. doi:10.1074/jbc.M504320200
27. Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO (2009) Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity 31(6):873–884. doi:10.1016/j.immuni.2009.09.018
28. Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, Davies DR (2008) Structural basis of Toll-like receptor 3 signaling with double-stranded RNA. Science 320(5874):379–381. doi:10.1126/science.1155406
29. Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO (2009) The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature 458(7242):1191–1195. doi:10.1038/nature07830
30. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124(4):783–801. doi:10.1016/j.cell.2006.02.015
31. Matsushima N, Tanaka T, Enkhbayar P, Mikami T, Taga M, Yamada K, Kuroki Y (2007) Comparative sequence analysis of leucine-rich repeats (LRRs) within vertebrate Toll-like receptors. BMC Genomics 8:124. doi:10.1186/1471-2164-8-124
32. Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, Sher A (2005) TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science 308(5728):1626–1629. doi:10.1126/science.1109893
33. Koblansky AA, Jankovic D, Oh H, Hieny S, Sungnak W, Mathur R, Hayden MS, Akira S, Sher A, Ghosh S (2013) Recognition of profilin by Toll-like receptor 12 is critical for host resistance to *Toxoplasma gondii*. Immunity 38(1):119–130. doi:10.1016/j.immuni.2012.09.016
34. Mathur R, Oh H, Zhang D, Park SG, Seo J, Koblansky A, Hayden MS, Ghosh S (2012) A mouse model of *Salmonella typhi* infection. Cell 151(3):590–602. doi:10.1016/j.cell.2012.08.042
35. Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, Ghosh S (2004) A Toll-like receptor that prevents infection by uro-

pathogenic bacteria. Science 303(5663):1522–1526. doi:10.1126/science.1094351

36. Oldenburg M, Kruger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, Bathke B, Lauterbach H, Suter M, Dreher S, Koedel U, Akira S, Kawai T, Buer J, Wagner H, Bauer S, Hochrein H, Kirschning CJ (2012) TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. Science 337(6098):1111–1115. doi:10.1126/science.1220363
37. Godfroy JI, Roostan M, Moroz YS, Korendovych IV, Yin H (2012) Isolated Toll-like receptor transmembrane domains are capable of oligomerization. PLoS One 7(11), e48875. doi:10.1371/journal.pone.0048875
38. Bowie A, O'Neill LA (2000) The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products. J Leukoc Biol 67(4):508–514
39. O'Neill LA, Golenbock D, Bowie AG (2013) The history of Toll-like receptors—redefining innate immunity. Nat Rev Immunol 13(6):453–460. doi:10.1038/nri3446
40. Kawai T, Akira S (2007) Signaling to NF-kappaB by Toll-like receptors. Trends Mol Med 13(11):460–469. doi:10.1016/j.molmed.2007.09.002
41. Savitsky D, Tamura T, Yanai H, Taniguchi T (2010) Regulation of immunity and oncogenesis by the IRF transcription factor family. Cancer Immunol Immunother 59(4):489–510. doi:10.1007/s00262-009-0804-6
42. Lin SC, Lo YC, Wu H (2010) Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. Nature 465(7300):885–890. doi:10.1038/nature09121
43. Kawasaki T, Kawai T (2014) Toll-like receptor signaling pathways. Front Immunol 5:461. doi:10.3389/fimmu.2014.00461
44. Israel A (2010) The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harb Perspect Biol 2(3):a000158. doi:10.1101/cshperspect.a000158
45. Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ (2001) TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 412(6844):346–351. doi:10.1038/35085597
46. Eliopoulos AG, Dumitru CD, Wang CC, Cho J, Tsichlis PN (2002) Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals. EMBO J 21(18):4831–4840
47. Banerjee A, Gugasyan R, McMahon M, Gerondakis S (2006) Diverse Toll-like receptors utilize Tpl2 to activate extracellular signal-regulated kinase (ERK) in hemopoietic cells. Proc Natl Acad Sci U S A 103(9):3274–3279. doi:10.1073/pnas.0511113103
48. Takeuchi O, Kaufmann A, Grote K, Kawai T, Hoshino K, Morr M, Muhlradt PF, Akira S (2000) Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a Toll-like receptor 2- and MyD88-dependent signaling pathway. J Immunol 164(2):554–557
49. Schnare M, Holt AC, Takeda K, Akira S, Medzhitov R (2000) Recognition of CpG DNA is mediated by signaling pathways dependent on the adaptor protein MyD88. Curr Biol 10(18):1139–1142
50. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410(6832):1099–1103. doi:10.1038/35074106
51. Kaisho T, Takeuchi O, Kawai T, Hoshino K, Akira S (2001) Endotoxin-induced maturation of MyD88-deficient dendritic cells. J Immunol 166(9):5688–5694
52. Adachi O, Kawai T, Takeda K, Matsumoto M, Tsutsui H, Sakagami M, Nakanishi K, Akira S (1998) Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity 9(1):143–150
53. Sims JE, Smith DE (2010) The IL-1 family: regulators of immunity. Nat Rev Immunol 10(2):89–102. doi:10.1038/nri2691
54. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413(6857):732–738. doi:10.1038/35099560
55. Kawai T, Adachi O, Ogawa T, Takeda K, Akira S (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 11(1):115–122
56. Kawai T, Takeuchi O, Fujita T, Inoue J, Muhlradt PF, Sato S, Hoshino K, Akira S (2001) Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol 167(10):5887–5894
57. Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T (2003) TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induc-

tion. Nat Immunol 4(2):161–167. doi:10.1038/ni886

58. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S (2003) Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. Science 301(5633):640–643. doi:10.1126/science.1087262
59. Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, Akira S (2002) Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. J Immunol 169(12):6668–6672
60. Kawai T, Akira S (2007) TLR signaling. Semin Immunol 19(1):24–32. doi:10.1016/j.smim.2006.12.004
61. Carty M, Goodbody R, Schroder M, Stack J, Moynagh PN, Bowie AG (2006) The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. Nat Immunol 7(10):1074–1081. doi:10.1038/ni1382
62. Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, Shimada N, Ohba Y, Takaoka A, Yoshida N, Taniguchi T (2005) IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 434(7034):772–777. doi:10.1038/nature03464
63. Cao W, Manicassamy S, Tang H, Kasturi SP, Pirani A, Murthy N, Pulendran B (2008) Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires the rapamycin-sensitive PI(3)K-mTOR-p70S6K pathway. Nat Immunol 9(10):1157–1164. doi:10.1038/ni.1645
64. Kawai T, Sato S, Ishii KJ, Coban C, Hemmi H, Yamamoto M, Terai K, Matsuda M, Inoue J, Uematsu S, Takeuchi O, Akira S (2004) Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol 5(10):1061–1068. doi:10.1038/ni1118
65. Shinohara ML, Lu L, Bu J, Werneck MB, Kobayashi KS, Glimcher LH, Cantor H (2006) Osteopontin expression is essential for interferon-alpha production by plasmacytoid dendritic cells. Nat Immunol 7(5):498–506. doi:10.1038/ni1327
66. Honda K, Ohba Y, Yanai H, Negishi H, Mizutani T, Takaoka A, Taya C, Taniguchi T (2005) Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. Nature 434(7036):1035–1040. doi:10.1038/nature03547
67. Tsujimura H, Tamura T, Kong HJ, Nishiyama A, Ishii KJ, Klinman DM, Ozato K (2004) Toll-like receptor 9 signaling activates NF-kappaB through IFN regulatory factor-8/IFN consensus sequence binding protein in dendritic cells. J Immunol 172(11):6820–6827
68. Tailor P, Tamura T, Kong HJ, Kubota T, Kubota M, Borghi P, Gabriele L, Ozato K (2007) The feedback phase of type I interferon induction in dendritic cells requires interferon regulatory factor 8. Immunity 27(2):228–239. doi:10.1016/j.immuni.2007.06.009
69. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, Kano S, Honda K, Ohba Y, Mak TW, Taniguchi T (2005) Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature 434(7030):243–249. doi:10.1038/nature03308
70. Negishi H, Fujita Y, Yanai H, Sakaguchi S, Ouyang X, Shinohara M, Takayanagi H, Ohba Y, Taniguchi T, Honda K (2006) Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. Proc Natl Acad Sci U S A 103(41):15136–15141. doi:10.1073/pnas.0607181103
71. Sancho D, Reis e Sousa C (2012) Signaling by myeloid C-type lectin receptors in immunity and homeostasis. Annu Rev Immunol 30:491–529. doi:10.1146/annurev-immunol-031210-101352
72. Drummond RA, Brown GD (2013) Signalling C-type lectins in antimicrobial immunity. PLoS Pathog 9(7), e1003417. doi:10.1371/journal.ppat.1003417
73. Zelensky AN, Gready JE (2005) The C-type lectin-like domain superfamily. FEBS J 272(24):6179–6217. doi:10.1111/j.1742-4658.2005.05031.x
74. Hoving JC, Wilson GJ, Brown GD (2014) Signalling C-type lectin receptors, microbial recognition and immunity. Cell Microbiol 16(2):185–194. doi:10.1111/cmi.12249
75. Iborra S, Sancho D (2015) Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors. Immunobiology 220(2):175–184. doi:10.1016/j.imbio.2014.09.013
76. Geijtenbeek TB, Gringhuis SI (2009) Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol 9(7):465–479. doi:10.1038/nri2569
77. Dambuza IM, Brown GD (2015) C-type lectins in immunity: recent developments. Curr Opin Immunol 32C:21–27. doi:10.1016/j.coi.2014.12.002

78. Gringhuis SI, Kaptein TM, Wevers BA, Theelen B, van der Vlist M, Boekhout T, Geijtenbeek TB (2012) Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1beta via a noncanonical caspase-8 inflammasome. Nat Immunol 13(3):246–254. doi:10.1038/ni.2222
79. Hise AG, Tomalka J, Ganesan S, Patel K, Hall BA, Brown GD, Fitzgerald KA (2009) An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. Cell Host Microbe 5(5):487–497. doi:10.1016/j.chom.2009.05.002
80. Gross O, Poeck H, Bscheider M, Dostert C, Hannesschlager N, Endres S, Hartmann G, Tardivel A, Schweighoffer E, Tybulewicz V, Mocsai A, Tschopp J, Ruland J (2009) Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. Nature 459(7245):433–436. doi:10.1038/nature07965
81. del Fresno C, Soulat D, Roth S, Blazek K, Udalova I, Sancho D, Ruland J, Ardavin C (2013) Interferon-beta production via Dectin-1-Syk-IRF5 signaling in dendritic cells is crucial for immunity to *C. albicans*. Immunity 38(6):1176–1186. doi:10.1016/j.immuni.2013.05.010
82. Dorhoi A, Desel C, Yeremeev V, Pradl L, Brinkmann V, Mollenkopf HJ, Hanke K, Gross O, Ruland J, Kaufmann SH (2010) The adaptor molecule CARD9 is essential for tuberculosis control. J Exp Med 207(4):777–792. doi:10.1084/jem.20090067
83. Zamze S, Martinez-Pomares L, Jones H, Taylor PR, Stillion RJ, Gordon S, Wong SY (2002) Recognition of bacterial capsular polysaccharides and lipopolysaccharides by the macrophage mannose receptor. J Biol Chem 277(44):41613–41623. doi:10.1074/jbc.M207057200
84. Astarie-Dequeker C, N'Diaye EN, Le Cabec V, Rittig MG, Prandi J, Maridonneau-Parini I (1999) The mannose receptor mediates uptake of pathogenic and nonpathogenic mycobacteria and bypasses bactericidal responses in human macrophages. Infect Immun 67(2):469–477
85. Schulert GS, Allen LA (2006) Differential infection of mononuclear phagocytes by *Francisella tularensis*: role of the macrophage mannose receptor. J Leukoc Biol 80(3):563–571. doi:10.1189/jlb.0306219
86. Zhang SS, Park CG, Zhang P, Bartra SS, Plano GV, Klena JD, Skurnik M, Hinnebusch BJ, Chen T (2008) Plasminogen activator Pla of Yersinia pestis utilizes murine DEC-205 (CD205) as a receptor to promote dissemination. J Biol Chem 283(46):31511–31521. doi:10.1074/jbc.M804646200
87. Geijtenbeek TB, van Kooyk Y (2003) DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. Curr Top Microbiol Immunol 276:31–54
88. Hillaire ML, Nieuwkoop NJ, Boon AC, de Mutsert G, Vogelzang-van Trierum SE, Fouchier RA, Osterhaus AD, Rimmelzwaan GF (2013) Binding of DC-SIGN to the hemagglutinin of influenza A viruses supports virus replication in DC-SIGN expressing cells. PLoS One 8(2), e56164. doi:10.1371/journal.pone.0056164
89. Iborra S, Izquierdo HM, Martínez-López M, Blanco-Menéndez N, Reis e Sousa C, Sancho D (2012) The DC receptor DNGR-1 mediates cross-priming of CTLs during vaccinia virus infection in mice. J Clin Invest 122(5):1628–1643. doi:10.1172/JCI60660
90. Zelenay S, Keller AM, Whitney PG, Schraml BU, Deddouche S, Rogers NC, Schulz O, Sancho D, Reis e Sousa C (2012) The dendritic cell receptor DNGR-1 controls endocytic handling of necrotic cell antigens to favor cross-priming of CTLs in virus-infected mice. J Clin Invest 122(5):1615–1627. doi:10.1172/JCI60644
91. van Die I, van Vliet SJ, Nyame AK, Cummings RD, Bank CM, Appelmelk B, Geijtenbeek TB, van Kooyk Y (2003) The dendritic cell-specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. Glycobiology 13(6):471–478. doi:10.1093/glycob/cwg052
92. Ritter M, Gross O, Kays S, Ruland J, Nimmerjahn F, Saijo S, Tschopp J, Layland LE, Prazeres da Costa C (2010) *Schistosoma mansoni* triggers Dectin-2, which activates the Nlrp3 inflammasome and alters adaptive immune responses. Proc Natl Acad Sci U S A 107(47):20459–20464. doi:10.1073/pnas.1010337107
93. Osorio F, Reis e Sousa C (2011) Myeloid C-type lectin receptors in pathogen recognition and host defense. Immunity 34(5):651–664. doi:10.1016/j.immuni.2011.05.001
94. Goodridge HS, Shimada T, Wolf AJ, Hsu YM, Becker CA, Lin X, Underhill DM (2009) Differential use of CARD9 by dectin-1 in macrophages and dendritic cells. J Immunol 182(2):1146–1154
95. Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Wevers B, Bruijns SC, Geijtenbeek TB (2009) Dectin-1 directs T helper cell differentiation by controlling noncanonical

NF-kappaB activation through Raf-1 and Syk. Nat Immunol 10(2):203–213. doi:10.1038/ni.1692

96. Gringhuis SI, Wevers BA, Kaptein TM, van Capel TM, Theelen B, Boekhout T, de Jong EC, Geijtenbeek TB (2011) Selective C-Rel activation via Malt1 controls anti-fungal T(H)-17 immunity by dectin-1 and dectin-2. PLoS Pathog 7(1), e1001259. doi:10.1371/journal.ppat.1001259
97. Leibundgut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV, Schweighoffer E, Tybulewicz V, Brown GD, Ruland J, Reis e Sousa C (2007) Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat Immunol 8(6):630–638. doi:10.1038/ni1460
98. Leibundgut-Landmann S, Osorio F, Brown GD, Reis e Sousa C (2008) Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses. Blood 112(13):4971–4980. doi:10.1182/blood-2008-05-158469
99. Osorio F, LeibundGut-Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C (2008) DC activated via dectin-1 convert Treg into IL-17 producers. Eur J Immunol 38(12):3274–3281. doi:10.1002/eji.200838950
100. Goodridge HS, Simmons RM, Underhill DM (2007) Dectin-1 stimulation by *Candida albicans* yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. J Immunol 178(5):3107–3115
101. Sato K, Yang XL, Yudate T, Chung JS, Wu J, Luby-Phelps K, Kimberly RP, Underhill D, Cruz PD Jr, Ariizumi K (2006) Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses. J Biol Chem 281(50):38854–38866. doi:10.1074/jbc.M606542200
102. Gringhuis SI, den Dunnen J, Litjens M, van Het Hof B, van Kooyk Y, Geijtenbeek TB (2007) C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. Immunity 26(5):605–616. doi:10.1016/j.immuni.2007.03.012
103. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, Foy E, Loo YM, Gale M Jr, Akira S, Yonehara S, Kato A, Fujita T (2005) Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J Immunol 175(5):2851–2858
104. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 5(7):730–737. doi:10.1038/ni1087
105. Loo YM, Gale M Jr (2011) Immune signaling by RIG-I-like receptors. Immunity 34(5):680–692. doi:10.1016/j.immuni.2011.05.003
106. Szabo A, Magyarics Z, Pazmandi K, Gopcsa L, Rajnavolgyi E, Bacsi A (2014) TLR ligands upregulate RIG-I expression in human plasmacytoid dendritic cells in a type I IFN-independent manner. Immunol Cell Biol 92(8):671–678. doi:10.1038/icb.2014.38
107. Wu J, Chen ZJ (2014) Innate immune sensing and signaling of cytosolic nucleic acids. Annu Rev Immunol 32:461–488. doi:10.1146/annurev-immunol-032713-120156
108. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G (2006) 5′-Triphosphate RNA is the ligand for RIG-I. Science 314(5801):994–997. doi:10.1126/science.1132505
109. Baum A, Sachidanandam R, Garcia-Sastre A (2010) Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. Proc Natl Acad Sci U S A 107(37):16303–16308. doi:10.1073/pnas.1005077107
110. Chiu YH, Macmillan JB, Chen ZJ (2009) RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell 138(3):576–591. doi:10.1016/j.cell.2009.06.015
111. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441(7089):101–105. doi:10.1038/nature04734
112. Peisley A, Lin C, Wu B, Orme-Johnson M, Liu M, Walz T, Hur S (2011) Cooperative assembly and dynamic disassembly of MDA5 filaments for viral dsRNA recognition. Proc Natl Acad Sci U S A 108(52):21010–21015. doi:10.1073/pnas.1113651108
113. Seth RB, Sun L, Ea CK, Chen ZJ (2005) Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3.

Cell 122(5):669–682. doi:10.1016/j.cell.2005.08.012

114. Sun Q, Sun L, Liu HH, Chen X, Seth RB, Forman J, Chen ZJ (2006) The specific and essential role of MAVS in antiviral innate immune responses. Immunity 24(5):633–642. doi:10.1016/j.immuni.2006.04.004
115. Hou F, Sun L, Zheng H, Skaug B, Jiang QX, Chen ZJ (2011) MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. Cell 146(3):448–461. doi:10.1016/j.cell.2011.06.041
116. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 17:189–220. doi:10.1146/annurev.immunol.17.1.189
117. Curtsinger JM, Valenzuela JO, Agarwal P, Lins D, Mescher MF (2005) Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. J Immunol 174(8):4465–4469
118. Kersse K, Bertrand MJ, Lamkanfi M, Vandenabeele P (2011) NOD-like receptors and the innate immune system: coping with danger, damage and death. Cytokine Growth Factor Rev 22(5–6):257–276. doi:10.1016/j.cytogfr.2011.09.003
119. Chen G, Shaw MH, Kim YG, Nunez G (2009) NOD-like receptors: role in innate immunity and inflammatory disease. Annu Rev Pathol 4:365–398. doi:10.1146/annurev.pathol.4.110807.092239
120. Bryant CE, Monie TP (2012) Mice, men and the relatives: cross-species studies underpin innate immunity. Open Biol 2(4):120015. doi:10.1098/rsob.120015
121. Yuen B, Bayes JM, Degnan SM (2014) The characterization of sponge NLRs provides insight into the origin and evolution of this innate immune gene family in animals. Mol Biol Evol 31(1):106–120. doi:10.1093/molbev/mst174
122. Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, Flavell RA, Girardin SE, Godzik A, Harton JA, Hoffman HM, Hugot JP, Inohara N, Mackenzie A, Maltais LJ, Nunez G, Ogura Y, Otten LA, Philpott D, Reed JC, Reith W, Schreiber S, Steimle V, Ward PA (2008) The NLR gene family: a standard nomenclature. Immunity 28(3):285–287. doi:10.1016/j.immuni.2008.02.005
123. Barbe F, Douglas T, Saleh M (2014) Advances in Nod-like receptors (NLR) biology. Cytokine Growth Factor Rev 25(6):681–697. doi:10.1016/j.cytogfr.2014.07.001
124. Girardin SE, Boneca IG, Carneiro LA, Antignac A, Jehanno M, Viala J, Tedin K, Taha MK, Labigne A, Zahringer U, Coyle AJ, DiStefano PS, Bertin J, Sansonetti PJ, Philpott DJ (2003) Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science 300(5625):1584–1587. doi:10.1126/science.1084677
125. Tanabe T, Chamaillard M, Ogura Y, Zhu L, Qiu S, Masumoto J, Ghosh P, Moran A, Predergast MM, Tromp G, Williams CJ, Inohara N, Nunez G (2004) Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. EMBO J 23(7):1587–1597. doi:10.1038/sj.emboj.7600175
126. Zhao Y, Yang J, Shi J, Gong YN, Lu Q, Xu H, Liu L, Shao F (2011) The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature 477(7366):596–600. doi:10.1038/nature10510
127. Rayamajhi M, Zak DE, Chavarria-Smith J, Vance RE, Miao EA (2013) Cutting edge: mouse NAIP1 detects the type III secretion system needle protein. J Immunol 191(8):3986–3989. doi:10.4049/jimmunol.1301549
128. Yang J, Zhao Y, Shi J, Shao F (2013) Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. Proc Natl Acad Sci U S A 110(35):14408–14413. doi:10.1073/pnas.1306376110
129. Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440(7081):228–232. doi:10.1038/nature04515
130. McNeela EA, Burke A, Neill DR, Baxter C, Fernandes VE, Ferreira D, Smeaton S, El-Rachkidy R, McLoughlin RM, Mori A, Moran B, Fitzgerald KA, Tschopp J, Petrilli V, Andrew PW, Kadioglu A, Lavelle EC (2010) Pneumolysin activates the NLRP3 inflammasome and promotes proinflammatory cytokines independently of TLR4. PLoS Pathog 6(11), e1001191. doi:10.1371/journal.ppat.1001191
131. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440(7081):237–241. doi:10.1038/nature04516
132. Duncan JA, Bergstralh DT, Wang Y, Willingham SB, Ye Z, Zimmermann AG, Ting JP (2007) Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP

binding to mediate inflammatory signaling. Proc Natl Acad Sci U S A 104(19):8041–8046. doi:10.1073/pnas.0611496104

133. Ye Z, Lich JD, Moore CB, Duncan JA, Williams KL, Ting JP (2008) ATP binding by monarch-1/NLRP12 is critical for its inhibitory function. Mol Cell Biol 28(5):1841–1850. doi:10.1128/MCB.01468-07
134. Zurek B, Proell M, Wagner RN, Schwarzenbacher R, Kufer TA (2012) Mutational analysis of human NOD1 and NOD2 NACHT domains reveals different modes of activation. Innate Immun 18(1):100–111. doi:10.1177/1753425910394002
135. Perregaux D, Gabel CA (1994) Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. J Biol Chem 269(21):15195–15203
136. Schorn C, Frey B, Lauber K, Janko C, Strysio M, Keppeler H, Gaipl US, Voll RE, Springer E, Munoz LE, Schett G, Herrmann M (2011) Sodium overload and water influx activate the NALP3 inflammasome. J Biol Chem 286(1):35–41. doi:10.1074/jbc.M110.139048
137. Arlehamn CS, Petrilli V, Gross O, Tschopp J, Evans TJ (2010) The role of potassium in inflammasome activation by bacteria. J Biol Chem 285(14):10508–10518. doi:10.1074/jbc.M109.067298
138. Latz E, Xiao TS, Stutz A (2013) Activation and regulation of the inflammasomes. Nat Rev Immunol 13(6):397–411. doi:10.1038/nri3452
139. Steimle V, Otten LA, Zufferey M, Mach B (1993) Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell 75(1):135–146
140. Nagarajan UM, Bushey A, Boss JM (2002) Modulation of gene expression by the MHC class II transactivator. J Immunol 169(9):5078–5088
141. Mori-Aoki A, Pietrarelli M, Nakazato M, Caturegli P, Kohn LD, Suzuki K (2000) Class II transactivator suppresses transcription of thyroid-specific genes. Biochem Biophys Res Commun 278(1):58–62. doi:10.1006/bbrc.2000.3769
142. Meissner TB, Li A, Kobayashi KS (2012) NLRC5: a newly discovered MHC class I transactivator (CITA). Microbes Infect 14(6):477–484. doi:10.1016/j.micinf.2011.12.007
143. Chamaillard M, Hashimoto M, Horie Y, Masumoto J, Qiu S, Saab L, Ogura Y, Kawasaki A, Fukase K, Kusumoto S, Valvano MA, Foster SJ, Mak TW, Nunez G, Inohara N (2003) An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol 4(7):702–707. doi:10.1038/ni945
144. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278(11):8869–8872. doi:10.1074/jbc.C200651200
145. Coulombe F, Divangahi M, Veyrier F, de Leseleuc L, Gleason JL, Yang Y, Kelliher MA, Pandey AK, Sassetti CM, Reed MB, Behr MA (2009) Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. J Exp Med 206(8):1709–1716. doi:10.1084/jem.20081779
146. Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Memet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL (2004) Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. Nat Immunol 5(11):1166–1174. doi:10.1038/ni1131
147. Travassos LH, Carneiro LA, Girardin SE, Boneca IG, Lemos R, Bozza MT, Domingues RC, Coyle AJ, Bertin J, Philpott DJ, Plotkowski MC (2005) Nod1 participates in the innate immune response to *Pseudomonas aeruginosa*. J Biol Chem 280(44):36714–36718. doi:10.1074/jbc.M501649200
148. Girardin SE, Tournebize R, Mavris M, Page AL, Li X, Stark GR, Bertin J, DiStefano PS, Yaniv M, Sansonetti PJ, Philpott DJ (2001) CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive Shigella flexneri. EMBO Rep 2(8):736–742. doi:10.1093/embo-reports/kve155
149. Opitz B, Puschel A, Beermann W, Hocke AC, Forster S, Schmeck B, van Laak V, Chakraborty T, Suttorp N, Hippenstiel S (2006) *Listeria monocytogenes* activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1-dependent manner in endothelial cells. J Immunol 176(1):484–490
150. Lysenko ES, Clarke TB, Shchepetov M, Ratner AJ, Roper DI, Dowson CG, Weiser JN (2007) Nod1 signaling overcomes resistance of *S. pneumoniae* to opsonophagocytic killing. PLoS Pathog 3(8), e118. doi:10.1371/journal.ppat.0030118
151. Ratner AJ, Aguilar JL, Shchepetov M, Lysenko ES, Weiser JN (2007) Nod1 mediates cytoplasmic sensing of combinations of extracellular

bacteria. Cell Microbiol 9(5):1343–1351. doi:10.1111/j.1462-5822.2006.00878.x

152. Silva GK, Gutierrez FR, Guedes PM, Horta CV, Cunha LD, Mineo TW, Santiago-Silva J, Kobayashi KS, Flavell RA, Silva JS, Zamboni DS (2010) Cutting edge: nucleotide-binding oligomerization domain 1-dependent responses account for murine resistance against *Trypanosoma cruzi* infection. J Immunol 184(3):1148–1152. doi:10.4049/jimmunol.0902254
153. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, Flavell RA (2005) Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307(5710):731–734. doi:10.1126/science.1104911
154. Shaw MH, Reimer T, Sanchez-Valdepenas C, Warner N, Kim YG, Fresno M, Nunez G (2009) T cell-intrinsic role of Nod2 in promoting type 1 immunity to Toxoplasma gondii. Nat Immunol 10(12):1267–1274. doi:10.1038/ni.1816
155. Sabbah A, Chang TH, Harnack R, Frohlich V, Tominaga K, Dube PH, Xiang Y, Bose S (2009) Activation of innate immune antiviral responses by Nod2. Nat Immunol 10(10):1073–1080. doi:10.1038/ni.1782
156. Watanabe T, Kitani A, Murray PJ, Strober W (2004) NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 5(8):800–808. doi:10.1038/ni1092
157. Tada H, Aiba S, Shibata K, Ohteki T, Takada H (2005) Synergistic effect of Nod1 and Nod2 agonists with Toll-like receptor agonists on human dendritic cells to generate interleukin-12 and T helper type 1 cells. Infect Immun 73(12):7967–7976. doi:10.1128/IAI.73.12.7967-7976.2005
158. Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G (2001) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. J Biol Chem 276(7):4812–4818. doi:10.1074/jbc.M008072200
159. Voss E, Wehkamp J, Wehkamp K, Stange EF, Schroder JM, Harder J (2006) NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. J Biol Chem 281(4):2005–2011. doi:10.1074/jbc.M511044200
160. Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK (2003) CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 124(4):993–1000. doi:10.1053/gast.2003.50153
161. Uehara A, Fujimoto Y, Fukase K, Takada H (2007) Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. Mol Immunol 44(12):3100–3111. doi:10.1016/j.molimm.2007.02.007
162. Opitz B, Forster S, Hocke AC, Maass M, Schmeck B, Hippenstiel S, Suttorp N, Krull M (2005) Nod1-mediated endothelial cell activation by *Chlamydophila pneumoniae*. Circ Res 96(3):319–326. doi:10.1161/01.RES.0000155721.83594.2c
163. Rosenstiel P, Fantini M, Brautigam K, Kuhbacher T, Waetzig GH, Seegert D, Schreiber S (2003) TNF-alpha and IFN-gamma regulate the expression of the NOD2 (CARD15) gene in human intestinal epithelial cells. Gastroenterology 124(4):1001–1009. doi:10.1053/gast.2003.50157
164. Kim YG, Park JH, Shaw MH, Franchi L, Inohara N, Nunez G (2008) The cytosolic sensors Nod1 and Nod2 are critical for bacterial recognition and host defense after exposure to Toll-like receptor ligands. Immunity 28(2):246–257. doi:10.1016/j.immuni.2007.12.012
165. Pudla M, Kananurak A, Limposuwan K, Sirisinha S, Utaisincharoen P (2011) Nucleotide-binding oligomerization domain-containing protein 2 regulates suppressor of cytokine signaling 3 expression in *Burkholderia pseudomallei*-infected mouse macrophage cell line RAW 264.7. Innate Immun 17(6):532–540. doi:10.1177/1753425910385484
166. Barnich N, Aguirre JE, Reinecker HC, Xavier R, Podolsky DK (2005) Membrane recruitment of NOD2 in intestinal epithelial cells is essential for nuclear factor-kappa B activation in muramyl dipeptide recognition. J Cell Biol 170(1):21–26. doi:10.1083/jcb.200502153
167. Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Nunez G, Inohara N (2008) A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J 27(2):373–383. doi:10.1038/sj.emboj.7601962
168. Watanabe T, Asano N, Fichtner-Feigl S, Gorelick PL, Tsuji Y, Matsumoto Y, Chiba T, Fuss IJ, Kitani A, Strober W (2010) NOD1 contributes to mouse host defense against *Helicobacter pylori* via induction of type I IFN and activation of the ISGF3 signaling pathway. J Clin Invest 120(5):1645–1662. doi:10.1172/JCI39481
169. Hitotsumatsu O, Ahmad RC, Tavares R, Wang M, Philpott D, Turer EE, Lee BL, Shiffin N, Advincula R, Malynn BA, Werts C,

Ma A (2008) The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. Immunity 28(3):381–390. doi:10.1016/j.immuni.2008.02.002

170. LeBlanc PM, Yeretssian G, Rutherford N, Doiron K, Nadiri A, Zhu L, Green DR, Gruenheid S, Saleh M (2008) Caspase-12 modulates NOD signaling and regulates antimicrobial peptide production and mucosal immunity. Cell Host Microbe 3(3):146–157. doi:10.1016/j.chom.2008.02.004
171. Fritz JH, Le Bourhis L, Sellge G, Magalhaes JG, Fsihi H, Kufer TA, Collins C, Viala J, Ferrero RL, Girardin SE, Philpott DJ (2007) Nod1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. Immunity 26(4):445–459. doi:10.1016/j.immuni.2007.03.009
172. Pavot V, Rochereau N, Resseguier J, Gutjahr A, Genin C, Tiraby G, Perouzel E, Lioux T, Vernejoul F, Verrier B, Paul S (2014) Cutting edge: new chimeric NOD2/TLR2 adjuvant drastically increases vaccine immunogenicity. J Immunol 193(12):5781–5785. doi:10.4049/jimmunol.1402184
173. Conti BJ, Davis BK, Zhang J, O'Connor W Jr, Williams KL, Ting JP (2005) CATERPILLER 16.2 (CLR16.2), a novel NBD/LRR family member that negatively regulates T cell function. J Biol Chem 280(18):18375–18385. doi:10.1074/jbc.M413169200
174. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, Davis BK, Allen IC, Holl EK, Ye Z, Rahman AH, Conti BJ, Eitas TK, Koller BH, Ting JP (2012) The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-kappaB. Nat Immunol 13(9):823–831. doi:10.1038/ni.2378
175. Zhang L, Mo J, Swanson KV, Wen H, Petrucelli A, Gregory SM, Zhang Z, Schneider M, Jiang Y, Fitzgerald KA, Ouyang S, Liu ZJ, Damania B, Shu HB, Duncan JA, Ting JP (2014) NLRC3, a member of the NLR family of proteins, is a negative regulator of innate immune signaling induced by the DNA sensor STING. Immunity 40(3):329–341. doi:10.1016/j.immuni.2014.01.010
176. Neerincx A, Lautz K, Menning M, Kremmer E, Zigrino P, Hosel M, Buning H, Schwarzenbacher R, Kufer TA (2010) A role for the human nucleotide-binding domain, leucine-rich repeat-containing family member NLRC5 in antiviral responses. J Biol Chem 285(34):26223–26232. doi:10.1074/jbc.M110.109736
177. Meissner TB, Li A, Biswas A, Lee KH, Liu YJ, Bayir E, Iliopoulos D, van den Elsen PJ, Kobayashi KS (2010) NLR family member NLRC5 is a transcriptional regulator of MHC class I genes. Proc Natl Acad Sci U S A 107(31):13794–13799. doi:10.1073/pnas.1008684107
178. Cui J, Zhu L, Xia X, Wang HY, Legras X, Hong J, Ji J, Shen P, Zheng S, Chen ZJ, Wang RF (2010) NLRC5 negatively regulates the NF-kappaB and type I interferon signaling pathways. Cell 141(3):483–496. doi:10.1016/j.cell.2010.03.040
179. Kumar H, Pandey S, Zou J, Kumagai Y, Takahashi K, Akira S, Kawai T (2011) NLRC5 deficiency does not influence cytokine induction by virus and bacteria infections. J Immunol 186(2):994–1000. doi:10.4049/jimmunol.1002094
180. Allen IC, Moore CB, Schneider M, Lei Y, Davis BK, Scull MA, Gris D, Roney KE, Zimmermann AG, Bowzard JB, Ranjan P, Monroe KM, Pickles RJ, Sambhara S, Ting JP (2011) NLRX1 protein attenuates inflammatory responses to infection by interfering with the RIG-I-MAVS and TRAF6-NF-kappaB signaling pathways. Immunity 34(6):854–865. doi:10.1016/j.immuni.2011.03.026
181. Rebsamen M, Vazquez J, Tardivel A, Guarda G, Curran J, Tschopp J (2011) NLRX1/NOD5 deficiency does not affect MAVS signalling. Cell Death Differ 18(8):1387. doi:10.1038/cdd.2011.64
182. Soares F, Tattoli I, Wortzman ME, Arnoult D, Philpott DJ, Girardin SE (2013) NLRX1 does not inhibit MAVS-dependent antiviral signalling. Innate Immun 19(4):438–448. doi:10.1177/1753425912467383
183. Xia X, Cui J, Wang HY, Zhu L, Matsueda S, Wang Q, Yang X, Hong J, Songyang Z, Chen ZJ, Wang RF (2011) NLRX1 negatively regulates TLR-induced NF-kappaB signaling by targeting TRAF6 and IKK. Immunity 34(6):843–853. doi:10.1016/j.immuni.2011.02.022
184. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES (2009) AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 458(7237):509–513. doi:10.1038/nature07710
185. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA (2009) AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature

458(7237):514–518. doi:10.1038/nature07725

186. Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, Hardy LL, Garceau V, Sweet MJ, Ross IL, Hume DA, Stacey KJ (2009) HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science 323(5917):1057–1060. doi:10.1126/science.1169841
187. Hansen JD, Vojtech LN, Laing KJ (2011) Sensing disease and danger: a survey of vertebrate PRRs and their origins. Dev Comp Immunol 35(9):886–897. doi:10.1016/j.dci.2011.01.008
188. Ishikawa H, Barber GN (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455(7213):674–678. doi:10.1038/nature07317
189. Ouyang S, Song X, Wang Y, Ru H, Shaw N, Jiang Y, Niu F, Zhu Y, Qiu W, Parvatiyar K, Li Y, Zhang R, Cheng G, Liu ZJ (2012) Structural analysis of the STING adaptor protein reveals a hydrophobic dimer interface and mode of cyclic di-GMP binding. Immunity 36(6):1073–1086. doi:10.1016/j.immuni.2012.03.019
190. Tanaka Y, Chen ZJ (2012) STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. Sci Signal 5(214), ra20. doi:10.1126/scisignal.2002521
191. Chen H, Sun H, You F, Sun W, Zhou X, Chen L, Yang J, Wang Y, Tang H, Guan Y, Xia W, Gu J, Ishikawa H, Gutman D, Barber G, Qin Z, Jiang Z (2011) Activation of STAT6 by STING is critical for antiviral innate immunity. Cell 147(2):436–446. doi:10.1016/j.cell.2011.09.022
192. Sun L, Wu J, Du F, Chen X, Chen ZJ (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339(6121):786–791. doi:10.1126/science.1232458
193. Zhang X, Shi H, Wu J, Zhang X, Sun L, Chen C, Chen ZJ (2013) Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. Mol Cell 51(2):226–235. doi:10.1016/j.molcel.2013.05.022
194. Gao D, Wu J, Wu YT, Du F, Aroh C, Yan N, Sun L, Chen ZJ (2013) Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science 341(6148):903–906. doi:10.1126/science.1240933
195. Li XD, Wu J, Gao D, Wang H, Sun L, Chen ZJ (2013) Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. Science 341(6152):1390–1394. doi:10.1126/science.1244040
196. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Eitson JL, Mar KB, Richardson RB, Ratushny AV, Litvak V, Dabelic R, Manicassamy B, Aitchison JD, Aderem A, Elliott RM, Garcia-Sastre A, Racaniello V, Snijder EJ, Yokoyama WM, Diamond MS, Virgin HW, Rice CM (2014) Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature 505(7485):691–695. doi:10.1038/nature12862