

Identification and functions of pattern-recognition receptors

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Since the identification of Toll-like receptors, our knowledge about pattern-recognition receptors (PRRs) has increased rapidly. Classes of PRRs that have been recently discovered include RIG-I-like receptors, Nod-like receptors, and C-type lectin receptors. Recent studies have started to clarify the molecular basis of PRR-ligand interactions, yet the numbers of PRRs and their ligands continue to increase. New technologies have elucidated the network regulation of immune responses at the cellular and *in vivo* levels. We review the most recent discoveries about PRRs and their ligands, their roles in intracellular and *in vivo* regulation of immune responses, and the systems biology of innate immunity. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Pattern-recognition receptor, Toll-like receptor, RIG-I-like receptor, Nod-like receptor, C-type lectin receptor, systems biology

Mammals have 2 types of immune systems: adaptive and innate immunity. Innate immunity was considered to be the less complex and less flexible system until the mid-1990s, when Toll was discovered in *Drosophila* species and its mammalian homologues, the Toll-like receptors (TLRs), were found to mediate recognition of pathogens by the innate immune system. These findings led to the discovery of pattern-recognition receptors (PRRs) and their roles in regulation of the entire immune system. Antigen-presenting cells, such as dendritic cells (DCs), use PRRs to recognize specific molecular signatures on pathogens called pathogen-associated molecular patterns (PAMPs). The recognition of PAMPs by PRRs leads to antigen-presenting cell activation and elicits adaptive immunity. Other endogenous molecules called danger-associated molecular patterns can also induce immune responses. There are several classes of PRRs: TLRs, RIG-I-like receptors, Nod-like receptors (NLRs), and

Abbreviations used

CARD domain:	Caspase recruitment domain
CLR:	C-type lectin receptor
DC:	Dendritic cell
ds:	Double-stranded
MyD88:	Myeloid differentiation primary response gene 88
NF:	Nuclear factor
NLR:	Nod-like receptor
PAMP:	Pathogen-associated molecular pattern
poly(I:C):	Polyinosinic-polycytidylic acid
PRR:	Pattern-recognition receptor
RLR:	RIG-I-like receptor
ROS:	Reactive oxygen species
ss:	Single-stranded
STING:	Stimulator of interferon genes
TLR:	Toll-like receptor
TXNIP:	Thioredoxin-interacting protein

C-type lectin receptors (CLRs). The list of PRRs and their ligands continues to expand. Molecular biology techniques, new cell biology approaches, and methods for *in vivo* observation of the spatiotemporal regulation of immunity have been used to elucidate complex host-pathogen interactions. We review the most recent findings about PRRs, the molecular mechanisms of ligand recognition, the spatial regulation of PRR-mediated immune responses, and the systems biology of the innate immune response.

TLR SIGNALING, LIGANDS, AND CELL BIOLOGY

In the innate immune system TLRs recognize different PAMPs (reviewed by Akira et al¹). This recognition leads to the activation of a genetic program through transcription factors, such as nuclear factor (NF) κ B, interferon regulatory factors, and activator protein 1, as well as posttranscriptional regulation of mRNA. Zfp36 (also known as TTP) and its homologues Zfp3611, Zfp3612, and Zfp3613 are CCCH-type zinc finger proteins that bind and destabilize mRNAs of some immune response genes, such as *Tnf* and *Csfl*.² Zc3h12a, another CCCH zinc finger protein, has an RNase moiety in its N-terminus and targets *Il6* and *Il12b* mRNAs.³ These zinc finger proteins are induced on TLR stimulation and regulate innate immune responses by controlling mRNA stability. A zinc finger protein of another class, Zcchc11, has been reported to control cytokine expression through uridylation of microRNAs that target cytokine genes.⁴ Posttranscriptional regulation and control of mRNA stability, rather than transcription, have been proposed to be the key regulators of certain immune pathways, such as the TNF-induced response⁵; these posttranscriptional regulation processes require further study to fully understand immune responses.

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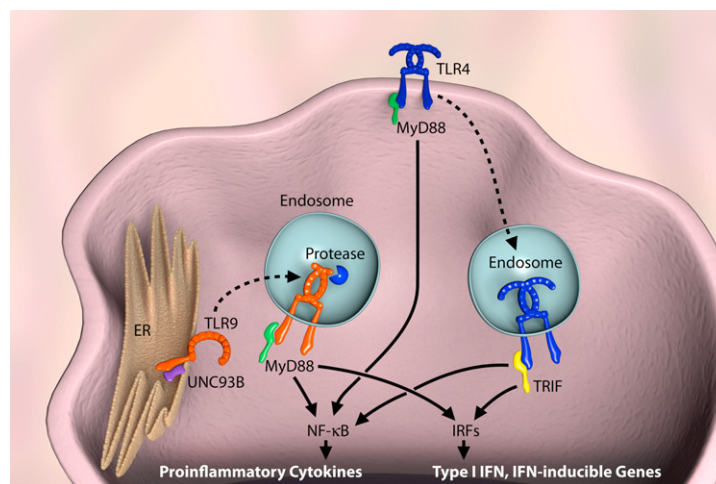


FIG 1. Cell biology of TLR signaling. TLR4 is located at the plasma membrane and translocates to the endosomal compartment on stimulation. Although MyD88-dependent signaling occurs without endosomal translocation of TLR4, TRIF-dependent signaling requires dynamin-dependent translocation.¹³ In a resting cell TLR9 is localized at the endoplasmic reticulum (ER) but is translocated to the endosome on stimulation, where a protease or proteases cleave TLR9. This process requires UNC93B, which associates with TLR9. After translocation, TLR9 transmits its signal through MyD88. *IRF*, Interferon regulatory factor; TRIF, Toll/IL-1 receptor domain containing adaptor inducing interferon- β .

Most PRR ligands fall into one of 2 classes. The first class consists of a bacterial cell-wall component that is recognized by TLR4 and TLR2. The second class includes nucleic acids from bacteria or viruses. TLR3 recognizes polyinosinic-polycytidylic acid (poly[I:C]), a viral double-stranded (ds) RNA analogue and possibly dsRNA. Crystallographic studies revealed the molecular basis of ligand recognition by the heterodimeric complexes TLR1-TLR2 and TLR2-TLR6,^{6,7} the homodimer TLR4–Myeloid differentiation protein-2 (MD-2),⁸ and the TLR3 homodimer.⁹ The interaction between TLR1 and TLR2 forms a pocket for its ligand, triacylated lipoprotein. Biologically active LPS contains 6 acyl chains; 5 of these are buried inside the pocket between TLR4 and MD-2, and the remaining chain is exposed at the surface of MD-2, possibly interacting hydrophobically with the TLR4 surface. In contrast, the TLR4 antagonists lipid IVa and eritoran have only 4 acyl chains and do not displace the phosphorylated glucosamine backbone exposed on the outside of MD-2, leading to defective activation of subsequent intracellular signaling.¹⁰

Although the crystal structures for TLR7, TLR8, and TLR9 have not been determined, it has been revealed that TLR7 and TLR8 recognize the antiviral drug imidazoquinoline and viral single-stranded (ss) RNA, whereas TLR9 recognizes bacterial and viral DNA, which are abundant in unmethylated CpG motifs.¹ TLR7 recognizes bacterial RNA in some cell types. There is controversy about how TLRs recognize nonself but not self nucleotides. The localization of TLR9 in endosome might prevent TLR9 from accidental exposure to self-DNA and subsequent activation.¹¹ The backbone structure of a nucleotide, rather than its sequence, might determine activation of TLR9, as well as TLR7.¹² Crystallographic studies are needed to reveal the mode of ligand recognition by TLR7 and TLR9.

TLR2 and TLR4 localize to the cell membrane, which allows them to detect bacteria outside of cells. After ligation with its ligand, TLR4 translocates from the cytoplasmic membrane to the endosome by means of a dynamin-dependent mechanism

(Fig 1).¹³ This translocation is important to elicit the Toll/IL-1 receptor domain containing adaptor inducing IFN- β (TRIF)-dependent pathway because Tumor necrosis factor receptor associated factor 3 (TRAF3), a scaffold protein, is localized at the endosome.¹³ Spatial regulation of PRRs appears to be important for proper signaling.

Unlike TLR4, the TLRs that recognize nucleic acids (TLR3, TLR7, and TLR9) reside in the endosome, where they can recognize viruses. After ligand recognition, these TLRs translocate to different compartments. TLR9 localizes to the endoplasmic reticulum of resting cells, but after ligand stimulation, it colocalizes with CpG-DNA and myeloid differentiation primary response gene 88 (MyD88) in the lysosome in an MyD88-independent manner (Fig 1).¹⁴ UNC93B has been identified as a regulator of translocation of TLR3, TLR7, and TLR9.^{15,16} The D34A mutation in UNC93B accelerates TLR7 signaling but suppresses TLR9 signaling.¹⁷ However, it is not clear how TLR9 transmits signals to UNC93B.

TLR9 is delivered to the lysosome, where it is believed to be cleaved by lysosomal endopeptidases. Deletion of lysosomal peptidases, such as cathepsin K¹⁸ or cathepsin B,¹⁹ abrogates TLR9 signaling, indicating their involvement in TLR9 cleavage. Asparagine endopeptidase-deficient cells are unable to cleave TLR9.²⁰ Interestingly, asparagine endopeptidase is recruited after TLR9 stimulation, indicating the importance of spatial regulation of signaling molecules.

LIGANDS FOR RIG-I-LIKE RECEPTOR AND FUNCTIONS

TLRs can recognize viruses that are in the endosomal compartment (ie, “outside” the cell) and elicit antiviral responses, such as production of type I interferon. However, viruses usually replicate inside the cell, and therefore an intracellular receptor or receptors are required for efficient antiviral immunity. RIG-I and the homologues Melanoma differentiation associated gene 5

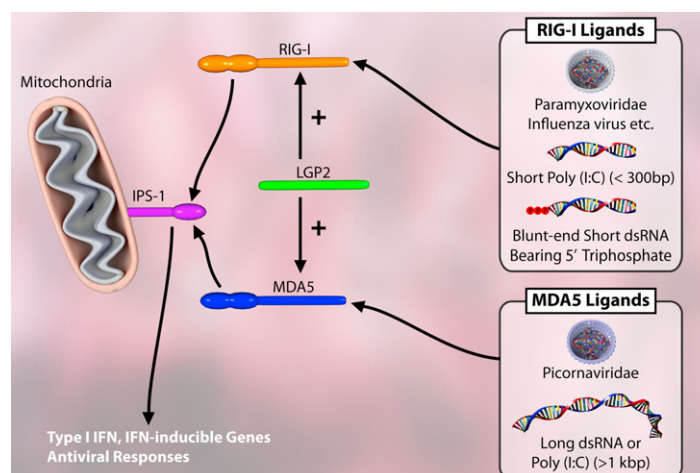


FIG 2. RIG-I and MDA5 ligands and signaling. RIG-I and MDA5 recognize the ligands presented in Table I; LGP2 positively regulates RIG-I and MDA5 signaling. These RLRs transmit signals through the mitochondria-localized adaptor molecule IPS-1 to activate expression of type I interferon (*IFN*) and *IFN*-inducible genes and the antiviral response.²¹

TABLE I. PRRs, PAMPs, and the signaling pathways they activate

PRRs	PAMPs	Signaling pathway activated
TLRs¹		
TLR1-TLR2	Triacylated lipopeptide	MyD88-dependent activation of NF- κ B
TLR2-TLR6	Diacylated lipopeptide	MyD88-dependent activation of NF- κ B
TLR4	LPS	MyD88-dependent activation of NF- κ B and TRIF-dependent activation of NF- κ B and IRF3
TLR3	Poly(I:C)	TRIF-dependent activation of NF- κ B and IRF3
TLR7	ssRNA	MyD88-dependent activation of NF- κ B and IRFs
TLR9	CpG-DNA	MyD88-dependent activation of NF- κ B and IRFs
RLRs²¹		
RIG-I	Paramyxoviridae, short blunt dsRNA bearing a 5' triphosphate (<50 bp), ²⁴ and short poly(I:C) (<300 bp) ²³	IPS-1-dependent activation of NF- κ B and IRFs and inflammasome activation
MDA5	Picornaviridae, long dsRNA, and long poly(I:C) ²³	IPS-1-dependent activation of NF- κ B and IRFs
LGP2	?	Positively regulating RLR signaling
NLRs^{1, 25}		
NOD2	Muramyl dipeptide	RIP2-dependent activation of NF- κ B
NALP3	Uric acid crystal, silica, asbestos, hemozoin, zymosan, <i>C albicans</i> , influenza virus, <i>L monocytogenes</i> , <i>S aureus</i>	Inflammasome activation
IPAF	Flagellin, <i>Salmonella typhimurium</i> , <i>Legionella pneumophila</i> , <i>Shigella flexneri</i>	Inflammasome activation
AIM2	dsDNA	Inflammasome activation
CLRs³⁰		
Mincle	SAP130 nuclear protein, ²⁶ <i>Malassezia</i> species, ²⁸ trehalose dimycolate ²⁹	Syk-dependent signaling
Clec9a/DNGR-1	Necrotic cells ²⁷	Syk-dependent signaling

(MDA5) and LGP2 (RIG-I-like receptors [RLRs]) are among the intracellular receptors for RNA viruses (Fig 2 and Table I).^{1,21-30} The receptors transmit their signal through a common adaptor protein, Interferon promoter stimulator-1 (IPS-1), to induce type I interferon production and antiviral responses.²¹

Unlike RIG-I and MDA5, which have caspase recruitment domains (CARD domains) in their N-terminal region and transmit signals through homotypic interaction with the CARD domain of IPS-1, LGP2 has no CARD domain. LGP2 is a positive regulator of the MDA5-mediated immune response,^{22,31} although the role of LGP2 in the RIG-I-mediated response is controversial.

Studies of mice with the mutation K30A in LGP2 demonstrated that the adenosine triphosphatase activity in the LGP2 helicase is required for LGP2 function; cells with this mutation did not respond to viruses that are recognized by either MDA5 or RIG-I.²² This indicates a positive role for LGP2 in RLR signaling.

Because RLRs have a helicase domain, it is highly presumable that RLR recognizes viral genomic RNA. RIG-I was first identified as a detector of poly(I:C). Subsequent studies revealed that RIG-I recognizes ssRNA that has a triphosphate moiety in its 5' terminus, although poly(I:C) can contain a RIG-I ligand.^{32,33} However, studies have shown that short dsRNA is a genuine

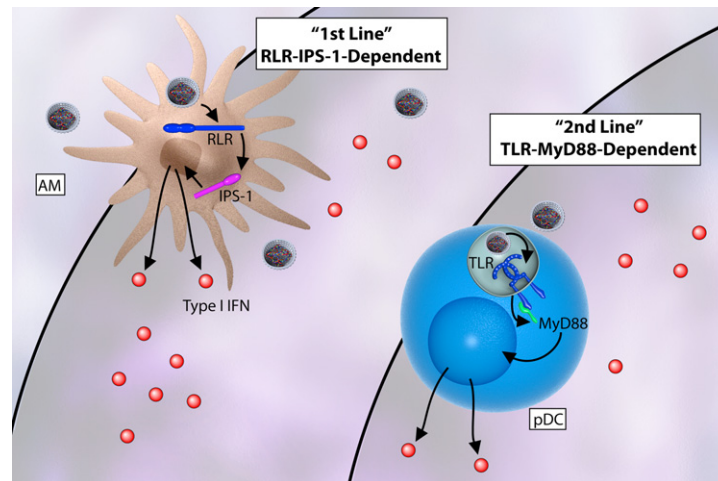


FIG 3. The pulmonary antiviral system.³⁵ Alveolar macrophages (AM) signal through RLR and IPS-1 to induce type I interferon (IFN), as the first line of defense against RNA viruses in the lung. If this system is disrupted by viruses, plasmacytoid DCs (pDC) signal through the TLR-MyD88 pathway to induce antiviral responses, such as type I IFN production, as a second line of immune defense.

ligand of RIG-I. RNase III–digested, relatively short poly(I:C) (approximately 300 bp) is also a RIG-I ligand, even in the absence of a 5' triphosphate.²³ Another report indicated that short, blunt-end dsRNA (<50 bp) bearing a 5' triphosphate is a RIG-I ligand.²⁴ This short dsRNA might be produced during replication of some negative-stranded ssRNA viruses, and therefore RIG-I can sense such viruses. MDA5 recognizes long poly(I:C) and long dsRNA, which is produced during replication of sense-strand ssRNA viruses, such as picornaviruses (Fig 2).^{23,34}

Taken together, there are 2 classes of receptors for viruses that induce an antiviral response that includes production of type I interferon. Do they work simultaneously in a cell, or do they work in a cell type–specific manner? On infection with RNA viruses, several cell types, such as conventional DCs, plasmacytoid DCs, and embryonic fibroblasts, respond to the infection and produce type I interferon. Several reports supported the concept that the TLR and RLR pathways work in parallel on viral infection *in vivo*. A study using a murine strain in which IFN- α 6 expression was monitored confirmed that plasmacytoid and conventional DCs and macrophages are responsible for type I interferon production in response to systemic RNA virus infection.³⁵ Plasmacytoid and conventional DCs use TLRs and RLRs differently *in vivo*. In plasmacytoid DCs interferon feedback signaling blocks viral replication and consequently masks the requirement for viral replication for type I interferon production.³⁶ Another study showed that vesicular stomatitis virus replicates inside plasmacytoid DCs, which sense the replicating viruses through TLR- and autophagy-dependent mechanisms. On deletion of Atg5, which is important in autophagy (a bulk protein degradation system), plasmacytoid DCs failed to produce type I interferon after viral infection. Plasmacytoid DCs therefore have different mechanisms for detecting different viruses.³⁷

Alveolar macrophages have also been shown to produce type I interferons in an RLR signaling-dependent manner during pulmonary infection with viruses.³⁵ On abrogation of the RLR pathway by IPS-1 deficiency, plasmacytoid DCs start to produce type I interferon. This indicates that in contrast to systemic infection, there are 2 innate antiviral systems in the lung: the alveolar macrophage–RLR system as a first line of defense and the

plasmacytoid DC–TLR system as a backup mechanism (Fig 3).³⁵ Consistently, alveolar macrophages control infection with viruses such as vaccinia virus and respiratory syncytial virus.^{38,39}

In contrast to the roles of RLRs in the innate immune response, their role in the adaptive immune response still remains to be clarified. Several reports indicated that the TLR-MyD88 pathway is required to elicit acquired immune responses. Lymphocytoid choriomeningitis virus, an ssRNA virus that belongs to the Arenaviridae family, induced innate and adaptive immunity in a MyD88-dependent manner.⁴⁰ Intranasal influenza virus infection induced MyD88- and IPS-1–dependent innate immune responses, but antibody production and T-cell memory depended only on the TLR7-MyD88 pathway.⁴¹ In contrast, on infection with respiratory syncytial virus, antibody production depended on either MyD88 or IPS-1.⁴² These results indicate that use of PRRs for the activation of adaptive immunity differs between viruses; further investigation is required to determine how the innate immune response elicits adaptive immune responses through different PRRs.

RECOGNITION OF INTRACELLULAR DNA

In contrast to receptors for RNA viruses, receptors for intracellular DNA viruses that induce type I interferon production have not been identified. Z-DNA binding protein 1 (ZBP1) (also called DAI/DLM1) is believed to be a receptor for intracellular DNA,⁴³ although there are many questions about its function (Fig 4).^{44–47} A possible downstream molecule, stimulator of interferon genes (STING), was identified in a functional screen.⁴⁸ The STING pathway was shown to be involved in IFN- β induction by DNA viruses, *Listeria monocytogenes*, and dsDNA.^{45,48} STING-deficient mice are susceptible to infection with herpes simplex virus, indicating the importance of this pathway in immunity against DNA viruses.⁴⁵ A recent report showed that nucleic acid–sensing receptors of the immune system are activated primarily through high-mobility group B proteins.⁴⁶ The identification of an intracellular DNA receptor will clarify the relationship between these molecules.

On stimulation, STING translocates from the endoplasmic reticulum to perinuclear vesicles.⁴⁵ Blocking this translocation

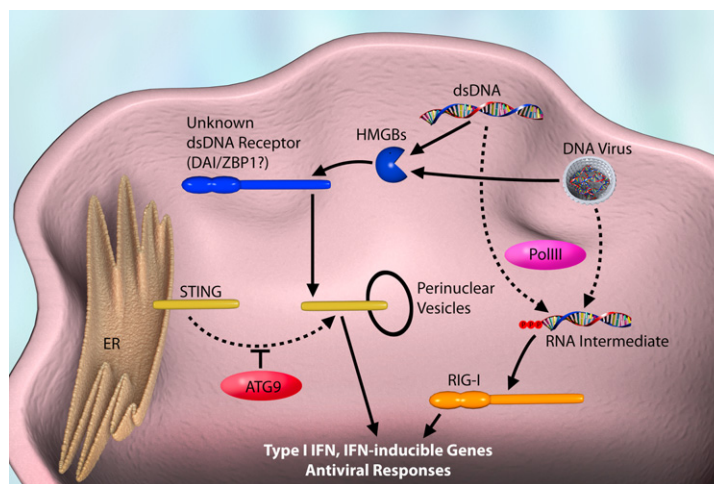


FIG 4. Recognition of intracellular exogenous dsDNA and DNA viruses. Double-stranded B-form DNA and DNA viruses are recognized by HMGB chromosomal proteins,⁴⁶ which activate an unidentified DNA receptor that signals through STING. On stimulation, STING translocates from the endoplasmic reticulum (ER) to the perinuclear membrane compartment, leading to production of type I interferon (IFN) and activation of IFN-inducible genes⁴⁵; translocation is inhibited by ATG9.⁴⁷ PolIII generates small RNA intermediates from intracellular DNA or DNA viruses, which is recognized by RIG-I to induce antiviral responses. HMGB, high mobility group box; PolIII, RNA polymerase III.

abrogates signaling, indicating the importance of spatial regulation of signaling molecules (Fig 4). Deletion of a component of Atg9 (a component of the autophagy machinery) promoted the translocation of STING.⁴⁷ Similar to TLR9 translocation on CpG DNA stimulation, the factors that induce the translocation of STING are not known.

Alternatively, recent studies indicated that RLRs are partially involved in the recognition of intracellular DNA through RNA polymerase III-mediated generation of ssRNA, which is a ligand for RLR under specific conditions (Fig 4).^{49–51} Further studies with infection models are required to reveal the importance of the RLR-mediated DNA recognition pathway in antiviral immunity.

SENSING PATHOGENS AND ENDOGENOUS LIGANDS: NLRs AND CLRs

A number of studies showed the importance of TLRs in the immune response against bacteria. Another class of PRRs, NLRs, have been identified as important sensors of bacterial infection. Nod1 and Nod2 are NLRs that recognize PAMPs derived from the bacterial cell wall. Unlike TLRs, Nod1 and Nod2 are localized to the cytoplasm and can elicit TLR-independent antibacterial responses.¹ Nod2 also serves as a sensor for respiratory syncytial virus.⁵² Moreover, recent reports demonstrated that Nod2 is involved in defense against the parasite *Toxoplasma gondii*.⁵³ Interestingly, in the antiparasite immunity Nod2 seems to have a T cell–intrinsic function rather than functions in macrophages or DCs.

Other NLRs, such as NALP1, NALP3, IPAF, and NAIP5, are components of a molecular complex called the inflammasome.²⁵ The inflammasome complex comprises one or some of the NLR proteins and caspase-1, which is activated in this complex and cleaves substrates, such as pro-IL-1 β and pro-IL-18, to produce mature proteins (Fig 5). The NALP3 inflammasome is activated by stimuli such as uric acid crystal, silica, and asbestos.²⁵ Interestingly, these compounds are crystals. Uric acid crystal is associated with gout. NALP3-deficient mice are resistant to uric acid

crystal-induced inflammation,⁵⁴ and thus NALP3 appears to be a receptor for danger-associated molecular patterns. Infections with the malaria parasite^{55–58} or the fungus *Candida albicans*^{59–61} were also reported to activate the NALP3 inflammasome. Activation involves hemozoin and zymosan particles (particularly β -glucan),⁶² respectively. Infection with *Listeria monocytogenes*, *Staphylococcus aureus*, or influenza virus also activates the NALP3 inflammasome, although the ligands from these pathogens that activate NALP3 have not been identified.

Recent reports indicated that activation of NALP3 does not result from its direct binding to the ligands but rather from destabilization of the lysosomal compartment, potassium efflux, and generation of reactive oxygen species (ROS).⁶³ It is possible that the stimuli mentioned above somehow induce one of these events and consequently activate the inflammasome. The IPAF and NAIP5 inflammasome is activated by bacterial flagellin (Fig 5).²⁵ However, direct binding of these NLRs to flagellin, as well as NALP3 to crystals, has not been proved. Uric acid directly binds to the cytoplasmic membrane, and the binding activates the Syk kinase pathway and thereafter activates the NALP3 inflammasome.⁶⁴ Deletion of *Atg16l1*, which encodes a gene involved in autophagy, increases production of ROS and IL-1 β on LPS stimulation.⁶⁵ A report showed that thioredoxin-interacting protein (TXNIP) dissociates from thioredoxin in an ROS-dependent manner, and the dissociated TXNIP binds to NALP3 and activates the NALP3 inflammasome.⁶⁶ Collectively, it seems that NALP3 and other NLRs are not really receptors for specific molecular patterns but sensors for the intracellular environment, such as ROS generation.

Intracellular nucleotides can activate the inflammasome. On vesicular stomatitis virus infection and stimulation with RNA bearing a 5' triphosphate, RIG-I is reported to be a receptor that induces not only antiviral responses but also caspase-1 activation through an adaptor molecule, apoptosis-associated speck-like protein containing a caspase-activating and recruitment domain (ASC) (Fig 5).⁶⁷ It will be interesting to determine whether this

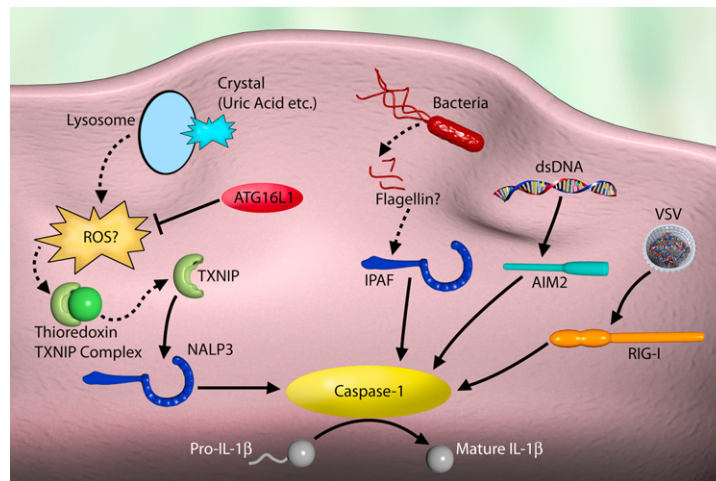


FIG 5. The inflammasome. Various NLRs, such as NALP3, IPAF, and AIM2 and possibly RIG-I, activate caspase-1, leading to cleavage of pro-IL-1 β to mature IL-1 β . Crystals destabilize lysosome, leading to ROS generation. NALP3 might sense such changes in the intracellular environment through TXNIP, which releases from the thioredoxin-TXNIP complex after generation of ROS. IPAF might recognize bacterial flagellin proteins. AIM2 binds to and recognizes dsDNA. VSV, vesicular stomatitis virus.

pathway also functions during infection with other viruses. Recently, AIM2 was identified as a DNA sensor that activates the inflammasome (Fig 5).⁶⁸⁻⁷¹ Although AIM2 is not a typical NLR, it shares an N-terminal signaling moiety, the pyrin domain, with some NLRs. AIM2 was shown to bind directly to its ligand, intracellular dsDNA, unlike other NLRs.

CLRs are also receptors for endogenous ligands. CLRs, such as Mincle²⁶ and Clec9a/DNGR-1,²⁷ recognize damaged but not apoptotic cells. Mincle detects Sin3-associated polypeptide p130 (SAP130), a component of small nuclear ribonucleoprotein, which is released from damaged cells. The ligand for Clec9a is an unknown molecule or molecules exposed on necrotic cells. Clec9a is expressed on CD8 α^+ DCs, which can cross-prime T cells through Clec9a. In this manner CLRs can regulate the acquired immune response.

Other CLRs, in addition to Mincle, serve as PRRs; dectin-1, dectin-2, and the mannose receptor recognize *C albicans*. The recognition of fungi by these receptors induces a T_H17 response.⁷²⁻⁷⁴ Mincle recognizes another fungus pathogen, *Malassezia* species, and provides antifungal immunity.²⁸ Mincle is also a PRR for trehalose dimycolate from *Mycobacterium tuberculosis*.²⁹ Similarly, many CLRs, such as DC-SIGN, langerin, Clec5a/MDL1, and mannose receptor, detect various pathogens and control both innate and adaptive immune responses through direct activation of immune signaling or acceleration of phagocytosis (reviewed by Geijtenbeek and Gringhuis³⁰). The role of CLRs in the immune response is becoming recognized; studies with knockout mice are needed to clarify their function.

SYSTEMS BIOLOGY OF INNATE IMMUNITY

The term *systems biology* does not yet have a clear definition, but if it is defined as methods to integrate comprehensive data on biological phenomena, computation, and even mathematics, some pioneering systems biology studies have been performed.

NF- κ B is an important transcription factor in immune responses that is well characterized as a physical system. Hoggmann et al⁷⁵ showed that the NF- κ B-I κ B signaling pathway

exhibits an oscillatory profile on TNF stimulation; oscillation of the concentration of active NF- κ B controls its transcriptional activity. A subsequent study suggested that the oscillation occurs in the translocation of NF- κ B to the nucleus.⁷⁶ Alternatively, LPS stimulation induced lasting, rather than oscillatory, activation of NF- κ B, probably because of feedback activation of NF- κ B by induced cytokines.^{77,78}

Gilchrist et al⁷⁹ used time-course microarray data and elementary mathematic modeling to show that the transcription factor activating transcription factor 3 is a negative regulator for TLR-induced responses. Other comprehensive gene expression studies indicated that at least 10 to more than 20 patterns of gene expression are observed based on clustering analyses.^{80,81} Scans of promoter regions of expressed genes revealed a large network of transcription factors, although a list of the specific factors in the network has not been obtained.

Despite the complexity, some studies have identified parts of the transcription factor circuit. The transcription factor CCAAT/enhancer-binding protein δ acts as an amplifier for TLR-mediated cytokine production and forms a feed-forward loop, along with NF- κ B,⁸² although the role of CCAAT/enhancer-binding protein δ is controversial.⁸¹ Another study attempted to construct a network picture of TLR signaling using data from DNA microarray analyses and gene knockdown studies.⁸³ They identified some candidate genes, such as a homeobox transcription factor, *Cbx4*, that might control TLR-induced gene expression. These types of integrated research studies are necessary to elucidate the immune signaling network.

CONCLUSION

The field of PRR research is expanding not only in the number of PRR molecules identified but also in the numbers of ligands. It seems almost impossible to unwind the tangled web of PRR-ligand interactions. Nonetheless, gaining a better picture of this network would improve our understanding of the entire immune response. Future studies might involve not only conventional molecular biology and genetic techniques but also new methods,

such as visualization of immune responses at the cell or tissue levels and systems biology approaches. Results from these types of studies will help us better identify and treat disease and immune disorders.

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