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REVIEW

Cellular and molecular regulation of innate inflammatory responses

Juan Liu[1] and Xuetao Cao[1,2]

Innate sensing of pathogens by pattern-recognition receptors (PRRs) plays essential roles in the innate discrimination between self and non-self components, leading to the generation of innate immune defense and inflammatory responses. The initiation, activation and resolution of innate inflammatory response are mediated by a complex network of interactions among the numerous cellular and molecular components of immune and non-immune system. While a controlled and beneficial innate inflammatory response is critical for the elimination of pathogens and maintenance of tissue homeostasis, dysregulated or sustained inflammation leads to pathological conditions such as chronic infection, inflammatory autoimmune diseases. In this review, we discuss some of the recent advances in our understanding of the cellular and molecular mechanisms for the establishment and regulation of innate immunity and inflammatory responses.
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INTRODUCTION

Innate immune system constitutes the first critical line against microbial infection by discriminating self and non-self components. The innate immune system relies on the host pattern-recognition receptors (PRRs) expressed by innate immune cells such as macrophages and dendritic cells (DCs) to rapidly recognize and respond to signals derived from the invading pathogens or injured self-cells. PRRs such as Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and nucleotide-binding domain and leucine-rich repeat containing molecules (NLRs) mediate the initial recognition of microbial components known as pathogen-associated molecular patterns (PAMPs).[1,2] This recognition triggers a series of signaling cascades that culminate in activation of transcriptional factors nuclear factor-κB (NF-κB), interferon regulatory factor (IRF) and activator protein-1 (AP-1), which induce numerous downstream genes encoding a broad range of inflammatory cytokines, chemokines, antimicrobial peptides, complement factors and interferons.[3,4]

TLRs are type I transmembrane molecules which transduce their downstream signaling through the MyD88-dependent pathway or the MyD88-independent but TRIF-dependent pathway, subsequently leading to activation of mitogen-activated protein kinase (MAPK), NF-κB and IRF pathway, inducing the production of proinflammatory cytokines and IFNs (Figure 1).[5–8] Innate immune activation of phagocytes through TLRs also induces an Mst1–Mst2–Rac signaling axis to activate intracellular microbicidal killing.[9,10]

RLRs, a family of cytoplasmic RNA helicases, are essential for innate recognition of viruses and are the key mechanism for the control of viral replication and dissemination. RIG-I[11] and melanoma differentiation-associated protein 5 (MDA5)[12] can recognize viral dsRNA and recruit the CARD containing adaptor protein MAVS (also known as IPS-1, CARDIF or VISA), leading to IRF activation and the production of type I IFN. In addition to RLRs, a group of cytosolic DNA sensors such as cyclic GMP-AMP synthase (cGAS),[13–15] absent in melanoma 2 (AIM2),[16,17] DDX41,[18,19] Rad50,[20,21] LRRFIP1,[22] DNA-dependent activator of IRFs (DAI),[23] as well as various RNA sensors such as IFN-induced protein with tetratricopeptide repeats 1 (IFIT1)[24] also play potent roles in inducing antiviral immune response, respectively via the adaptor protein

[1]National Key Laboratory of Medical Immunology & Institute of Immunology, Second Military Medical University, Shanghai 200433, China and [2]National Key Laboratory of Medical Molecular Biology, Department of Immunology & Center for Immunotherapy, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100005, China
Correspondence: Dr X Cao or Dr J Liu, National Key Laboratory of Medical Immunology & Institute of Immunology, Second Military Medical University, Shanghai 200433, China.
E-mail: caoxt@immunol.org or juanliu@immunol.org
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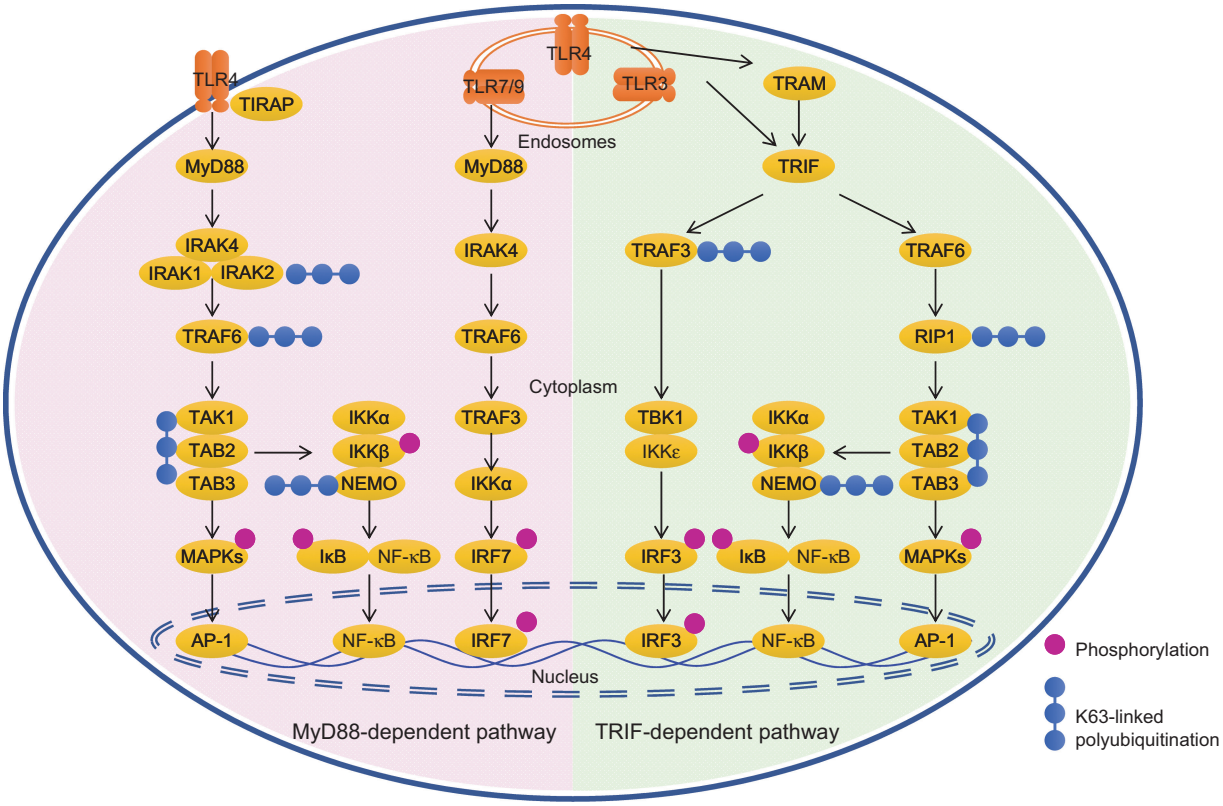

Figure 1 Schematic of signaling pathways of TLRs. Most TLRs with the exception of TLR3 initiate a MyD88-dependent pathway as shown in the left part. With the cooperation of Mal/TIRAP, TLR4 induces the MyD88–IRAK4 complex to recruit IRAK1 and IRAK2, which then interact with and induces K63-linked polyubiquitination of TRAF6 and TAK1/TAB2/TAB3 complexes. Activated TAK1 subsequently induce phosphorylation and activation of MAPKs and the IKK complex consisting of IKKα, IKKβ and K63-polyubiquitinated NEMO, finally promoting activation of AP-1 and NF-κB, and production of proinflammatory cytokines. TLR7 and TLR9 induces MyD88 activation to recruit signaling complex formed by IRAK4, TRAF6 and TRAF3, which induces phosphorylation and activation of IRF7 and production of type I IFN. Meanwhile, TLR3 and internalized TLR4 activate TRIF-dependent signaling as shown in the right part. TRAM is needed for the interaction between TLR4 and TRIF. TRIF recruits TRAF6 and RIP1, which induces downstream activation MAP kinases and NF-κB, similar to the MyD88-dependent pathway. TRIF also activates that TRAF3/TBK1/IKKε axis to promote IRF3-dependent expression of type I IFN.

stimulator of interferon genes (STING, also known as MITA, ERIS or MPYS) or the MAVS pathway. cGAS, which was previously thought to recognize cytoplasmic dsDNA over 40 bp in a sequence-independent manner, is recently shown to recognize unpaired guanosines flanking short (12–20 bp) dsDNA (Y-form DNA) found in human immunodeficiency virus type 1 to induce type I IFN production.[25,26] In neutrophils, transcription factor Sox2 could directly recognize microbial DNA through its high-mobility-group domain to activate innate immunity against microbial infection.[27–29]

NLRs consist of a large group of intracellular PRRs that includes NODs (nucleotide-binding oligomerization domains), NLRPs (LRR- and pyrin-domain (PYD)-containing protein), CIITA (Class II, major histocompatibility complex, transactivator), IPAF (ICE protease-activating factor) and NAIPs (neuronal apoptosis inhibitory protein), which vary in the effector domain they use to transduce downstream signals.[30,31] NOD1 and NOD2 respectively recognize meso-diaminopimelic acid (Meso-DAP) and muramyl dipeptide of intracellular bacteria to trigger host defense against bacterial infection via the MAPK and NF-κB pathway. NOD1 and NOD2 are also shown to recruit ATG16L1 to the plasma membrane at the site of bacterial entry to induce autophagy, a process which is critical for the limitation of bacterium invasion.[32,33]

Inflammasomes are large multi-molecular platforms that recruit the adaptor ASC to activate caspase-1, leading to the maturation and secretion of IL-18 and IL-1β and pyroptotic cell death, thus contributing to innate inflammatory responses to microbial or danger signals.[34,35] ASC particles could accumulate in the extracellular space to amplify activation of caspase-1 and maturation of IL-1β.[36,37] Recognition of dsDNA by AIM2 also recruits the inflammasome adaptor ASC to induce caspase-1-dependent inflammasome activation and IL--1β production. Activation of AIM2 inflammasome by *Francisella tularensis* subspecies novicida (*F. novicida*) is dependent on guanylate-binding proteins (GBPs)-mediated bacterium lysis for release of pathogenic DNA. GBPs are encoded by

interferon-stimulated genes which is induced by *F. novicida* via the DNA sensor cGAS and its adaptor STING.[38,39] In addition, RNA viral infection also triggers inflammasome activation and IL-1β production. Recognition of RNA virus by RIG-I mediates ASC/caspase-1 inflammasome-dependent IL-1β procession in a manner independent of MAVS and NLRP3.[40] RNA virus also initiates assembly of the receptor-interacting protein 1 (RIP1)–RIP3 complex to drive mitochondrial damage and activation of NLRP3 inflammasome via GTPase dynamin-related protein1 (DRP1).[41]

While efficient activation of PRR signaling is essential for the establishment of antimicrobial host defense and maintenance of tissue homeostasis, dysregulated or exaggerated innate immune response may cause pathological inflammation and even lead to pathogenesis of autoimmune diseases, inflammatory diseases, and cancer and so on. Thus, a delicate regulatory network is required to achieve the optimal signal output of innate immune responses, that is to efficiently eliminate invading pathogens while to avoid harmful immunological diseases. Comprehensive and multi-level mechanisms have evolved to tightly regulate the magnitude and duration of PRR signaling.[42,43] In this review, we summarize the molecular and cellular mechanisms underlying the activation and regulation of innate inflammatory responses.

MOLECULAR REGULATION OF INNATE IMMUNITY AND INFLAMMATION

Gene-specific regulation of inflammatory responses

PRR-triggered inflammatory responses involve the activation and suppression of several thousands of genes with distinct functions.[44] How to ensure the specific gene to be activated or silenced at the right time and space is a fundamental question in innate immune regulation. Epigenetic mechanisms such as DNA methylation, histone modifications and non-coding RNAs emerge to play essential roles in gene-specific transcriptional regulation of innate immunity via controlling chromatin status and gene expression.[45–47] These chromatin modifiers perform coordinated actions to convert the extracellular stimuli into the complex gene expression patterns during innate inflammatory responses. At the steady state, the poised/inactive enhancers are occupied by lineage-determining transcription factors known as pioneers, such as PU.1 and marked with a combination of H3K4me1 and repressive H3K27me3. Upon TLR stimulation, the pioneer transcription factor PU.1 allows the binding of signal-dependent transcription factors such as NF-κB, IRFs, AP-1, and STAT and relaxes chromatin structure with acquisition of H3K27ac and removal of H3K27me3 marks.[48,49] Notably, various specific enzymes or mediators have been shown to regulate inflammatory gene expression via controlling chromatin status. In the antiviral immunity, DNA methyltransferase Dnmt3a upregulates histone deacetylase 9 (HDAC9) via epigenetic mechanisms to deacetylases the kinase Tank-binding kinase 1 (TBK1) for activation, contributing to enhanced IFN production.[50] Viral infection also upregulates the expression of protein lysine methyltransferase Setdb2 to occupy and induce the repressive H3K9me3 of *Cxcl1* promoters. The decreased CXCL1 inhibits infiltration of neutrophils and thus mediate sensitivity to bacterial superinfection after infection with influenza virus. Thus, chromatin modification provide molecular basis for the crosstalk between inflammatory responses against different pathogens.[51] During the late phase of inflammatory response, induction of *de novo* 5-hydroxymethylation upstream of the *Il6* locus by a methylcytosine dioxygenase TET2 recruits HDAC2 to inhibit *Il6* transcription via histone deacetylation and is critical for termination of the high transcription of *Il6*.[52] It will be intriguing to further investigate the molecular mechanisms and physiological relevance of epigenetic modification at different phases of innate inflammatory response.

In addition, a number of nuclear receptors, such as glucocorticoid receptor,[53] peroxisome proliferator-activated receptor gamma,[54] liver X receptor,[55] small heterodimer partner (SHP),[56] nuclear receptor subfamily 4, group A, members (NR4A1)[57] and (NR4A2)[58] inhibit TLR4-induced NF-κB downstream genes via inhibiting NF-κB activity and/or enhancing the NCoR/SMRT corepressor complex to limit inflammatory gene programs. NR4A1 enhances host resistance to lipopolysaccharide (LPS) sepsis in mice via inhibiting NF-κB binding on target gene promoter regions. Interestingly, LPS-activated p38α blocks the suppressive activity of NR4A1 by inducing its phosphorylation and therefore facilitates LPS-induced inflammatory response. It is also shown that NR4A1 limits the production of norepinephrine in macrophages through recruitment of the CoREST–histone deacetylase complex to the *Th* promoter, thus inhibiting the pathogenesis of experimental autoimmune encephalomyelitis, outlining a novel molecular link between sympathetic stress response and inflammation.[59]

Signal-specific regulation of inflammatory responses

Post-translational modifications (PTMs) constitute an essential layer of regulation of innate inflammatory signaling via affecting the function and activity of existing signaling molecules at post-translational level.[60,61] The conventional PTMs such as ubiquitination and phosphorylation and unconventional PTMs such as methylation, acetylation and sumoylation target nearly all critical components of PRR signaling, such as receptors, adaptors, enzymes and transcriptional factors to modulate the quality of PRR signals.[62–65]

Taking PTM control of TLR-triggered tumor necrosis factor receptor-associated factor 6 (TRAF6)/NF-κB signaling pathway as an example. Removal of K63-linked polyubiquitination by A20[66] and TRAF family member-associated NF-κB activator (TANK)[67] is shown to modulate TRAF6 activity for inhibition of TLR signaling activation.[68–70] Besides, phosphorylation of TRAF6 by germinal center kinase MST4 prevents TRAF6 oligomerization and autoubiquitination and consequently inhibits inflammatory responses.[71] In addition, Rhbdd3, a member of rhomboid family of proteases, negatively regulates TLR-triggered activation of NF-κB and IL-6 production in DCs, contributing to balanced T-cell-mediated immunity and prevention of autoimmunity. Mechanistically, Rhbdd3 localizes in early endosomes in DCs and interacts with K27-linked

ubiquitination of NF-κB essential modifier (NEMO) and subsequently recruits A20 to facilitate A20-mediated K63-linked deubiquitination of NEMO.[72,73] By contrast, iRhom2, a novel noncatalytic relative of rhomboid proteins, facilitates LPS and *Listeria*-induced TNF production by promoting the TNF convertase (TACE) maturation and trafficking,[74,75] and also enhances innate immunity to DNA viruses by mediating STING trafficking and stability.[76] The differential regulation of innate immunity by Rhomboid family members awaits further investigation, and is likely to be related with their different subcellular localization that may provide specific biochemical and physical environment for specific signaling modifications.

Notably, the enzymes that mediate PTM control of PRR signaling, such as A20, are themselves being tightly controlled in a gene-specific manner, thus forming an intersecting network to modulate the quality of innate immune signaling. The transcriptional repressor downstream regulatory element antagonist modulator binds to the downstream regulatory elements (DREs) of A20 gene to repress A20 transcription, leading to enhancement of TLR-triggered NF-κB activation. By contrast, binding of the transcription factor USF1 to the DRE-associated E-box domain in the gene encoding A20 strengthens A20 expression in response to inflammatory stimuli.[77] In addition, histone methyltransferase Ash1l binds to the promoter regions of A20 gene to enhance the H3K4 methylation level, thus inducing A20 gene transcription and expression, consequently suppressing TLR signaling and inflammatory autoimmune diseases.[78] These studies further highlight the importance of crosstalk between PTM and chromatin modification in innate immune signaling regulation.

For the regulation of type I IFN-dependent antiviral immunity, protein PTM also plays an indispensable role via their effects in activity of signaling molecules such as RIG-I,[79] NEMO and IRF3. The tumor suppressor PTEN-mediated negative phosphorylation at Ser97 of IRF3 controls the import of IRF3 into the nucleus, contributing to inhibition of IRF3 activation and type I IFN production.[80,81] E3 ligase TRIM29 inhibits IRF3 signaling via the transcription factor NF-κB by directing binding to NEMO and inducing its ubiquitination and proteolytic degradation.[82] In addition, sumoylation contributes to repression of both inflammatory and antiviral responses, partially via targeting and suppressing activity of the *Ifnb1* promoter.[83] These data identify key negative regulators of innate immunity and might have important clinical implications for related inflammatory and infectious diseases.

Dysregulation of inflammasomes have been closely associated with diverse inflammatory diseases, therefore the negative regulation of inflammasomes are essential for prevention of excessive inflammation and maintenance of immune homeostasis.[84,85] PYD-only protein POP3 competes with ASC to bind AIM2-like receptors (ALRs), thus acting as an inhibitor of DNA virus-induced activation of ALR inflammasomes in monocytes and macrophages.[86,87] Neurotransmitter dopamine (DA)/dopamine D1 receptor (DRD1) signaling pathway negatively regulate NLRP3 inflammasome activation and NLRP3-dependent systemic inflammation via a second messenger cyclic adenosine monophosphate, which binds to NLRP3 and promotes its ubiquitination and degradation via the E3 ubiquitin ligase MARCH7.[88] Moreover, protein kinase A directly phosphorylates NLRP3 at Ser295 and attenuates its ATPase function, accordingly, mutations in NLRP3-encoding residues adjacent to Ser295 are linked to the auto-inflammatory disease cryopyrin-associated periodic syndromes (CAPS).[89] These studies reveal endogenous regulatory mechanisms of inflammasome regulation and suggest molecular basis and potential therapeutic targets of inflammatory and autoimmune diseases.

Interplay across different PRR signaling pathways

The TLRs, RLRs and NLRs trigger an intensively interacting network of downstream signaling to activate innate defense against invading pathogens (Figure 2). Distinct set of PRRs in different species, tissues, cells or cellular organelles display a partially overlapped and compensated recognition of PAMPs during inflammatory conditions. LPS from Gram-negative bacteria, is crucial for TLR4 activation, but also initiates innate immune signaling via detection by cytosol caspase-4/5/11 in mammals,[90,91] while in plants via sensing by transmembrane receptor kinase LORE. LORE confers recognition of LPS in plants and the subsequent induction of an immune response.[92,93] Whether additional proteins, e.g. LPS-binding protein is involved in LORE-mediated recognition of LPS remain to be elucidated.

A mutual inhibition of RLR and TLR pathways has been shown. RLR-triggered IRF3 following viral stimulation inhibits TLR-induced IRF5 activation following bacterial stimulation via dominantly occupying *Il12b* promoter, thus explaining the molecular mechanisms of bacterial superinfection post-viral infection.[94] Conversely, TLR7 small-molecule agonist inhibits nucleic acid-mediated TLR3, TLR7, TLR9 and RIG-I-dependent type I IFN signaling via inhibiting the formation of phosphorylated signal transducer and activator of transcription factor 1 (p-STAT1), p-STAT2 and IRF9 complex.[95]

Meanwhile, RIG-I plays a positive role in regulation of inflammasome and IL-1β secretion. Upon RNA viral stimulation, RIG-I could interact with adaptor ASC to trigger caspase-1-dependent inflammasome activation and IL-1β maturation.[34] On the contrary, some NLR members play negative roles in regulating RLR-mediated type I IFN responses via targeting distinct signaling molecules: for NLR family CARD domain-containing protein 5 (NLRC5) via interaction with RIG-I and MDA5;[96] for NLR family member X1 (NLRX1) via interaction with MAVS and disruption of RLR–MAVS interactions;[97,98] for NLRC3 via impeding the interaction between STING and TBK1 interaction;[99] for NLRP4 via targeting TBK1 for degradation.[100]

NOD proteins and TLRs are both critical for host defense against bacterial infection. NOD1 and NOD2 agonists play synergistic effects with TLR2, TLR3, TLR4, and TLR9 agonists to promote maturation and activation of DCs and basophils.[101–103] On the contrary, NOD2 deficiency increases TLR2-mediated activation of NF-κB and dysregulated TLR2 in

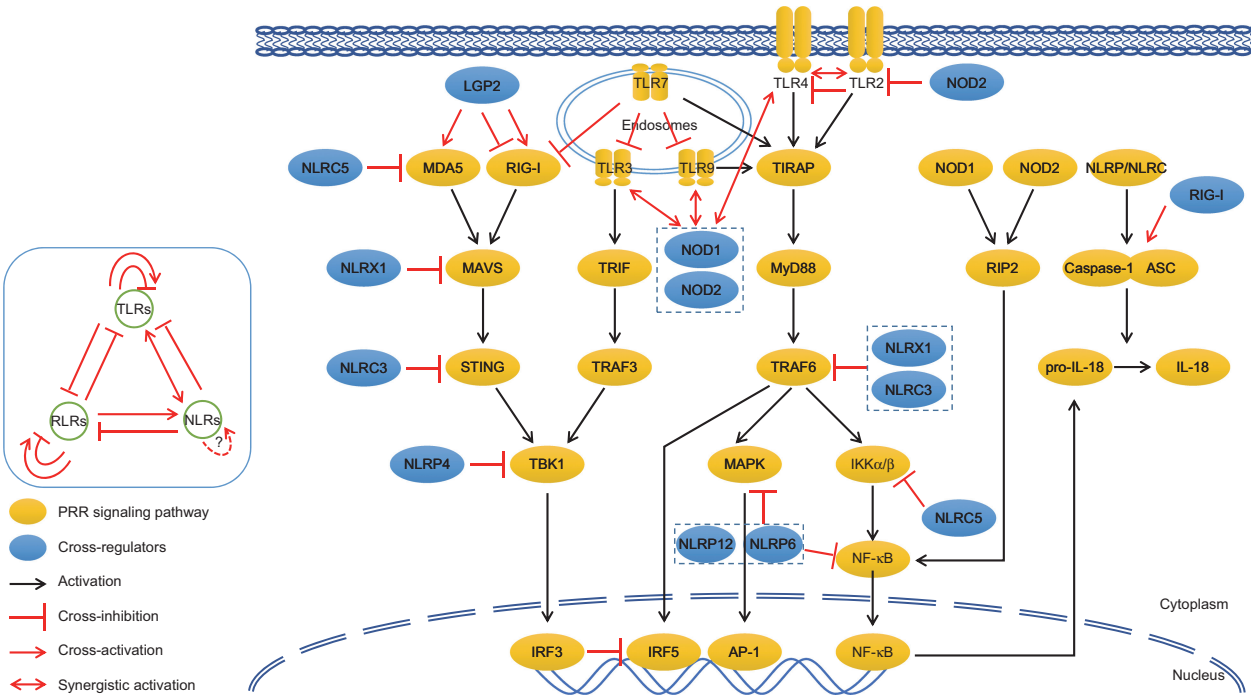

Figure 2 Interplay across TLR, RLR and NLR signaling pathways. TLR, RLR and NLR-triggered signaling pathways display complex interplay with each other. ① Crosstalk inside PRR family: LGP2 both positively and negatively regulate MDA5 and RIG-I signaling, so do TLR2 for TLR4. ② TLR-mediated cross-regulation: TLR7 agonists inhibit TLR3, TLR9 and RIG-I signaling; TLR3/4/9 synergize with NOD1/2 for amplified innate immune responses. ③ RLR-mediated cross-regulation: RIG-I signaling inhibits TLR signaling via competitive occupancy at *Il12* promoter by IRF5 over IRF3, but promotes NLRP3 inflammasome activation via interacting with ASC. ④ NLR-mediated cross-regulation: NOD2 tolerates TLR2 signaling for prevention of colitis. NLRX1, NLRC3/5, NLRP4/6/12 suppress TLR and RLR signaling via targeting distinct molecules.

NOD2-deficient mice causes the development of antigen-specific colitis.[104,105] Not only NOD2, many other NLRs play inhibitory roles in regulating the signaling of TLRs: for NLRX1 and NLRC3 via interfering with the TRAF6-NF-κB signaling;[61,106,107] for NLRC5 via interacting with and blocking phosphorylation of IκB kinase α (IKKα) and IKKβ;[59] for NRLP6 and NLRP12 via targeting MAPK and NF-κB activation;[108,109] for NLRC4 via downregulating TLR5-mediated antibody immune responses against flagellin.[110] These synergistic or antagonistic interactions across these three PRR families contribute to a cross-linked and finely tuned network of PRR signaling in response to the large repertoire of PAMPs and ensures the most effective and proper outcomes of innate immune responses.

CELLULAR REGULATION OF INNATE IMMUNITY AND INFLAMMATION

PRR-triggered inflammatory responses are mediated and regulated by a variety of immune and non-immune cells.[111,112] The endothelium system is critical player of innate defense via releasing inflammatory cytokines and chemokines in the first barrier to recruit monocytes, neutrophils, eosinophils and basophils from the circulation.[113–116] TLR stimulation induce phenotypic and functional maturation of DC into potent antigen-presenting cells which efficiently initiate and control adaptive immune responses, while in combination with IFN-γ could induce M1 macrophage polarization that is critical for phagocytosis and killing of invading pathogens. In this section, we focus on two important cell populations in innate immunity, DCs as the key bridge between innate and adaptive immunity, and innate lymphoid cells (ILCs) as the key mediator of effector responses during innate immunity.

Dendritic cells

DCs play important roles in the initiation and modulation of adaptive immune responses. DCs represent a complicated heterogeneous cell populations with distinct developmental origins, surface markers and effector/regulatory functions. Conventional DCs are further classified into subgroups according to the expression of CD8α that is regulated by a complex network of cytokines and transcriptional factors.[117–121] While $CD8\alpha^-$ classical dendritic cells (cDCs) primarily promote antigen-specific $CD4^+$ T-cell activation via the MHC II pathway, $CD8\alpha^+$ cDCs characteristically mediate cross-presentation of exogenous antigens on MHC I molecules to cytotoxic T lymphocytes (CTL). Ligation of TLRs by microbial products in DCs rapidly induces the expression of inflammatory cytokines, chemokines and chemokine receptors, co-stimulatory molecules and MHC molecules, allowing procession and presentation of antigens to T cells, and therefore play essential roles in

determining the activation and differentiation of T-cell subsets.[122–127] The activation of DCs by TLR agonists is accompanied by a rapid increase in glycolysis DC which is dependent on signaling via the kinases TBK1, IKKε and Akt. TLR-driven glycolytic flux serves an essential role in supporting the *de novo* synthesis of fatty acids for activation and function of DCs.[128,129]

Meanwhile, novel subsets of DC are also important for inducing immune tolerance toward harmless components via induction of immune tolerance under specific physiological or pathological conditions.[130] The regulatory capacity of DCs at steady state is programmed by specific local microenvironment, such as stromal cells of the spleen, lung and liver or intestinal epithelial cells.[131–135] Thymic DCs induce Treg-cell development via production of IL-2,[136] and that CD11b(−) cDCs from the gut-draining lymph nodes were critical for induction of peripheral Treg cells and oral tolerance.[137] The presence of exogenous immunosuppressive mediators, genetic manipulation, specific pathogenic stimuli also induce regulatory property of DCs through various mechanisms.[138]

The cross-presentation of exogenous antigens via MHC class I molecules to initiate CTL responses is essential for immunological defense against viruses, intracellular bacteria and tumors. While Rab11a activity recruits and keeps MHC-I within endosomal recycling compartment (ERC) under steady condition, MyD88-dependent TLR signals drive IKK2-mediated phosphorylation of phagosome-associated SNAP23, orchestrating ERC-phagosome fusion, promoting enrichment of phagosomes with ERC-derived MHC-I, and finally allowing cross-presentation during infection.[139] Transcription factor TFEB,[140] cell stress sensor IRE-1α,[141–143] NF-κB-inducing kinase (NIK)[144] and the lectin Siglec-G[145] have been shown to regulate DC cross-presentation in initiating antigen-specific CTL responses via distinct molecular mechanisms. For example, Siglec-G inhibits cross-presentation by CD8α+ DC via impairing the formation of the MHC class I-exogenous antigen peptide complex, contributing to suppression of CTL responses to intracellular bacterial infection with Listeria monocytogenes or Mycobacterium bovis bacillus Calmette–Guérin and tumors. Siglec-G is associated with SHP-1 to inhibit the activation of NOX2, consequently leading to promotion of phagosomal acidification and less effective antigen cross-presentation. This study provides new mechanistic insight into DC-mediated regulation of innate and adaptive immune responses under inflammatory conditions.

Innate lymphoid cells

ILCs are most recently identified populations of innate immune cells that play an important role in lymphoid tissue development, metabolic homeostasis and innate immunity against microbial infection and are increasingly linked with diverse pathological conditions such as infection, chronic inflammation, metabolic disease and cancer.[146,147] Distinct ILC groups are defined on the basis of expression of surface markers, transcription factors and cytokine secretion profiles, and effector functions in homeostasis and inflammation.[148] Whereas ILC1s and ILC3s are essential to host defense against infection by viruses, intracellular bacteria and parasites, ILC2s potently drive type 2 inflammation and mediate allergic inflammation, tissue repair and anti-helminth innate immunity.[149–153] A novel population of IL-25-responsive inflammatory ILC2 (iILC2) could develop into IL-33-responsive natural ILC2 (nILC2)-like cells and contribute to immunity to both helminths and fungi.[154,155] Inflammatory cytokines such as IL-1 and IFN,[156,157] and crosstalk with stromal cells, epithelial cells and various immune cells such as DC,[158] T cells[159] and B cells[160,161] are important for regulation of ILC function and plasticity at the crossroad of homeostasis and inflammation. For example, ILC3 can enhance antibody production by splenic marginal zone B cells via integrating stromal and myeloid signals. Further investigations are required to uncover the detailed mechanism underlying the plasticity and flexibility in the functions of distinct ILCs under biological and pathological conditions.

The cellular and molecular events that underlie ILC fate specification and functional regulation attract much attention in the past few years. Key transcription factors and regulators that control the development of ILC subsets at different stages are being increasingly identified, such as TOX, TCF-1, NFIL3, Id2, Runx3, GATA-3 and so on.[162–164] TOX-deficient mice have diminished numbers of LTi cells, NK cells, ILC1, ILC2 and ILC3 cells, indicating that TOX is required for *in vivo* differentiation of common lymphoid progenitors into ILC lineage-restricted cells.[165,166] Using novel reporter mice, researchers identified a novel subset of early ILC progenitors (EILPs) with distinctive expression of transcription factor TCF-1. EILPs exclusively and efficiently gives rise to NK cells and all known adult helper ILC lineages and therefore are perhaps the earliest ILC-committed progenitors identified so far.[167,168] The interaction of the TOX, TCF-1 and other transcription factors and epigenetic factors in the development of ILCs cells awaits further investigation.

CONCLUSIONS AND PERSPECTIVES

Increasing evidence reveal an essential role of PRRs in innate sensing of pathogens and initiation of innate inflammatory responses. A delicate regulatory network of PRR signaling both at molecular and cellular level contribute to an appropriate and effective host immune response under steady and inflammatory state. Though substantial progress have been made in depicting the initiation, activation and regulation of PRR-mediated innate immune response, some intriguing question still challenge further investigation. How does the immune system organize tissue-, cell- and gene-specific regulation of innate inflammatory responses? What is molecular basis for the combination, assembly and translocation of signaling molecule machinery? What is the developmental and functional characteristic of many other rare but important cell populations of innate immune systems, such as natural killer cells,[169] invariant natural killer T cells, mast cells, plasmacytoid DCs and so on.

Excitingly, with the rapid development in immunological technological platforms and combination of immunology and many disciplines such as epigenetics, genetics, biochemistry and neurobiology, substantial progress are being achieved in evaluating how the immune system quickly and accurately respond to external and internal stimuli. Single-cell sequencing technologies have identified the complex heterogeneities and divergences of cell phenotype and function.[170–172] Advances in genomics, transcriptomics, proteomics, metabolomics, interactomics, phenomics have illustrated the complex networks of immune cells and molecules in the settings of health and diseases.[173] Development of new mouse strains such as the mouse strain lacking iNKT cells[174] and new disease models such as the mice with myeloid-cell-specific deletion of A20 as a new model of rheumatoid arthritis[175] have provided useful tools for immunological studies. Cell fate mapping and *in vivo* imaging technologies have enabled real-time, dynamic and *in situ* assessment of the activity and function of immune system.[176-178] Future investigations will provide essential insights into the regulatory mechanisms of innate immunity and inflammation and outline potential clues for the development of effective therapeutic approaches of inflammatory diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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