Immunity
Review

Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity

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Toll-like receptors (TLRs) are germline-encoded pattern recognition receptors (PRRs) that play a central role in host cell recognition and responses to microbial pathogens. TLR-mediated recognition of components derived from a wide range of pathogens and their role in the subsequent initiation of innate immune responses is widely accepted; however, the recent discovery of non-TLR PRRs, such as C-type lectin receptors, NOD-like receptors, and RIG-I-like receptors, suggests that many aspects of innate immunity are more sophisticated and complex. In this review, we will focus on the role played by TLRs in mounting protective immune responses against infection and their crosstalk with other PRRs with respect to pathogen recognition.

Introduction

Recognition of microbial pathogens is an essential element for the initiation of innate immune responses such as inflammation and is mediated by germline-encoded pattern-recognition receptors (PRRs) that recognize molecular structures that are broadly shared by pathogens, known as pathogen-associated molecular patterns (PAMPs) (Janeway, 1989). Upon PAMP recognition, PRRs initiate a serious of signaling programs that execute the first line of host defensive responses necessary for killing infectious microbes. In addition, PRR signaling simultaneously induces maturation of dendritic cells (DCs), which is responsible for alerting induction of the second line of host defense, so-called adaptive immunity.

Toll-like receptors (TLRs) were the first PRRs to be identified. They are also the most well characterized and recognize a wide range of PAMPs (Akira et al., 2006; Beutler, 2009; Hoffmann, 2003; Medzhitov, 2007). TLRs are type I transmembrane proteins and comprise an ectodomain, which contains leucine-rich repeats that mediate the recognition of PAMPs, a transmembrane region, and cytosolic Toll-IL-1 receptor (TIR) domains that activate downstream signaling pathways. They are expressed either on the cell surface or associated with intracellular vesicles. To date, 10 and 12 functional TLRs have been identified in human and mouse, respectively. Each TLR detects distinct PAMPs derived from viruses, bacteria, mycobacteria, fungi, and parasites. These include lipoproteins (recognized by TLR1, TLR2, and TLR6), double-stranded (ds) RNA (TLR3), lipopolysaccharide (LPS) (TLR4), flagellin (TLR5), single-stranded (ss) RNA (TLR7 and TLR8), and DNA (TLR9) (Table 1; Akira et al., 2006). Upon recognition of respective PAMPs, TLRs recruit a specific set of adaptor molecules that harbor TIR domain, such as MyD88 and TRIF, and initiate downstream signaling events that leads to the secretion of inflammatory cytokines, type I IFN, chemokines, and antimicrobial peptides (Kawai and Akira, 2010). These responses cause recruitment of neutrophils, activation of macrophages, and induction of IFN-stimulated genes, resulting in direct killing of the infected pathogens. Moreover, activation of TLR signaling leads to maturation of DCs, contributing to the induction of adaptive immunity.

Although TLRs play a central role in the initiation of immune responses against a number of pathogens, it has become apparent that PRRs other than TLRs are also involved in PAMP recognition and the control of innate immunity (Table 1). These include membrane-bound C-type lectin receptors (CLRs), cytosolic proteins such as NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), and unidentified proteins that mediate sensing of cytosolic DNA or retrovirus infection (see reviews in this issue of *Immunity* by Osorio and Reis e Sousa, 2011; Loo and Gale, 2011; Elinav et al., 2011). CLRs are a large superfamily of membrane proteins comprising one or more C-type lectin-like domains, which largely elicit inflammatory responses by recognizing fungal and bacterial PAMPs. RLRs, which include RIG-I, MDA5, and LGP2, are RNA helicases that recognize RNA species released into the cytoplasm in a variety of cell types and coordinate antiviral programs via type I IFN induction. The NLR family comprises more than 20 members. NLRs are expressed intracellularly and respond to various PAMPs to trigger inflammatory responses. Several NLRs such as NALP1 (NLRP1) and NALP3 (NLRP3) form the inflammasomes along with ASC and Caspase-1 and mediate processing of pro-IL-1β to mature IL-1β for releases. DAI and IFI16 are candidate cytosolic dsDNA sensors and induce type I IFN production whereas AIM2 recognizes dsDNA and induces the secretion of IL-1β (Barbalat et al., 2011; Barber, 2011).

Intact microbial pathogens are usually composed of a number of PAMPs, which activate multiple PRRs. Moreover, different PRRs may recognize the same PAMP (Table 1). Hence, TLRs, in concert with other PRRs, orchestrate both pathogen-specific and cell type-specific host immune responses to fight infections. Here, we describe the role of TLRs in inducing innate immune responses and in shaping the adaptive immune responses to various pathogens, including bacteria, viruses, fungi, and protozoan parasites. The crosstalk between TLRs and other PRRs in mounting effective immune responses is also discussed.

Table 1. PAMP Detection by TLRs and Other PRRs

Species	PAMPs	TLR Usage	PRRs Involved in Recognition
Bacteria, mycobacteria	LPS	TLR4	
	lipoproteins, LTA, PGN, lipoarabinomannan	TLR2/1, TLR2/6	NOD1, NOD2, NALP3, NALP1
	flagellin	TLR5	IPAF, NAIP5
	DNA	TLR9	AIM2
	RNA	TLR7	NALP3
Viruses	DNA	TLR9	AIM2, DAI, IFI16
	RNA	TLR3, TLR7, TLR8	RIG-I, MDA5, NALP3
	structural protein	TLR2, TLR4	
Fungus	zymosan, β-glucan	TLR2, TLR6	Dectin-1, NALP3
	Mannan	TLR2, TLR4	
	DNA	TLR9	
	RNA	TLR7	
Parasites	tGPI-mutin (*Trypanosoma*)	TLR2	
	glycoinositolphospholipids (*Trypanosoma*)	TLR4	
	DNA	TLR9	
	hemozoin (*Plasmodium*)	TLR9	NALP3
	profilin-like molecule (*Toxoplasma gondii*)	TLR11	

Cellular Localization of TLRs

TLR1, TLR2, TLR4, TLR5, and TLR6 are localized on the cell surface and largely recognize microbial membrane components whereas TLR3, TLR7, TLR8, and TLR9 are expressed within intracellular vesicles and recognize nucleic acids (Blasius and Beutler, 2010). Recently, it was shown that TLR11, a relative of TLR5 expressed on the cell surface, is also expressed in intracellular compartments (Pifer et al., 2011). TLR13 is also expressed in intracellular vesicles although its cognate PAMP has not yet been identified (Blasius and Beutler, 2010).

Intracellular vesicles within DCs and other innate immune cells, in which TLR3, TLR7, TLR8, and TLR9 are localized, include the endoplasmic reticulum (ER), endosomes, lysosomes, and endolysosomes (Blasius and Beutler, 2010). The intracellular localization enables TLRs to recognize nucleic acids delivered to the intracellular compartments after the uptake of viruses and other pathogens or infected cells. By contrast, cellular nucleic acids present in the extracellular environment are rapidly degraded by nucleases and do not access these intracellular vesicles. Therefore, intracellular localization is important for avoiding contact with "self" nucleic acids, a process that would otherwise risk the initiation of autoimmune diseases. TLR3, TLR7, TLR8, and TLR9 are sequestered in the ER and are delivered to the endosomes (where they encounter and respond to their cognate PAMPs) via Golgi apparatus. Once inside the endosomes, the N-terminal region of the TLRs is processed by multiple lysosomal proteases, including cathepsins and asparagine endopeptidase, to generate functional receptors that elicit signaling (Blasius and Beutler, 2010; Ewald et al., 2011). UNC93B1 is a 12 membrane-spanning protein present in the ER and its mutation abrogates cytokine production and the upregulation of costimulatory molecules in response to TLR3, TLR7, and TLR9 ligands (Tabeta et al., 2006). UNC93B1 interacts with these TLRs and regulates their trafficking from the ER to the endosomal compartments (Figure 1; Brinkmann et al., 2007; Kim et al., 2008). UNC93B1 interacts more strongly with TLR9 than TLR7, resulting in a bias toward TLR9 sensing (Fukui et al., 2009). Although it is thought that TLR11 is expressed on the cell surface, it also interacts with UNC93B1 within the intracellular vesicles (Pifer et al., 2011). Thus, it is possible that UNC93B1 controls the mobilization of not only nucleic acid-sensing TLRs but also at least one protein-sensing TLR.

TLR mobilization is also controlled by other proteins within the ER, including PRAT4A and gp96. PRAT4A regulates the exit of TLR1, TLR2, TLR4, TLR7, and TLR9 from the ER and their respective trafficking to the plasma membrane and endososmes (Figure 1; Saitoh and Miyake, 2009). However, it does not influence TLR3 trafficking, which suggests that trafficking of TLR3 is differentially regulated from that of TLR7 and TLR9. gp96, a member of ER-resident heat-shock protein 90 family, functions as a general chaperone for most TLRs, including cell surface TLR1, TLR2, TLR4, and TLR5 and intracellular TLR7 and TLR9 (Saitoh and Miyake, 2009).

TLR Signaling Pathways

After recognizing their respective PAMPs, TLRs activate signaling pathways that provide specific immunological responses tailored to the microbes expressing that PAMP. The specific response initiated by individual TLRs depends on the recruitment of a single, or a specific combination of, TIR-domain-containing adaptor protein (e.g., MyD88, TIRAP, TRIF, or TRAM) (Figure 1; Kawai and Akira, 2010). MyD88 is utilized by all TLRs (with the exception of TLR3) and members of IL-1 receptor family and transmits signals culminating in NF-κB and MAP kinase activation and the induction of inflammatory cytokines. TLR3 and TLR4 use TRIF to activate an alternative pathway leading to the activation of NF-κB and IRF3 and the induction of type I IFN and inflammatory cytokine productions. TLR2 and TLR4 use TIRAP as an additional adaptor to recruit MyD88. TRAM acts as a bridge between TLR4 and TRIF.

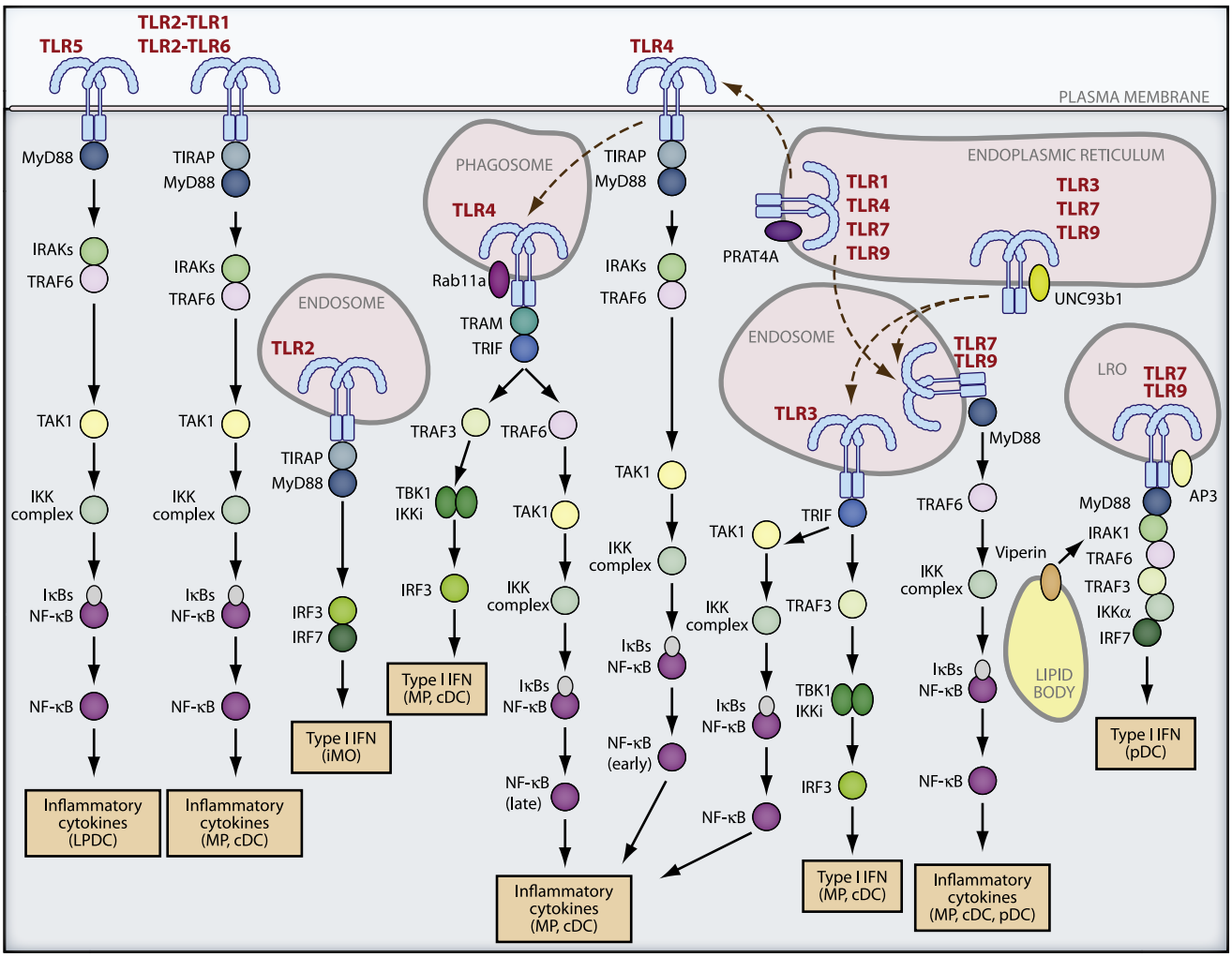

Figure 1. TLR Trafficking and Signaling

Individual TLRs initiate overlapping and distinct signaling pathways in various cell types such as macrophages (MP), conventinal DC (cDC), plasmacytoid DC (pDC), lamina propria DC (LPDC), and inflammatory monocytes (iMO). PAMP engagement induces conformational changes of TLRs that allow homo- or heterophilic interactions of TLRs and recruitment of adaptor proteins such as MyD88, TIRAP, TRIF, and TRAM. TLR5, which is highly expressed on the cell surface of LPDC, uses MyD88 and activates NF-κB through IRAKs, TRAF6, TAK1, and IKK complex, resulting in induction of inflammatory cytokines. Heterodimers of TLR1-TLR2 and TLR2-TLR6 are also expressed on the cell surface and induce NF-κB activation through recruitment of TIRAP and MyD88 in macrophages and cDCs. In iMO, TLR2 is found to be expressed within the endosome and induce type I IFN via IRF3 and IRF7 in response to viruses. TLR4, which is expressed on the cell surface, initially transmits signals for the early-phase activation of NF-κB by recruiting TIRAP and MyD88. TLR4 is then transported into Rab11a-positive phagosomes that contain bacteria, where it recruits TRAM and TRIF and activates TRAF3-TBK1-IRF3 axis as well as late-phase NF-κB activation for the induction of type I IFN. Both early- and late-phase activation of NF-κB is required for the induction of inflammatory cytokines. TLR3, TLR7, and TLR9 are localized mainly to the ER in the steady state and traffic to the endosomal compartment, where they engage with their ligands. UNC93B1, which interacts with these TLRs in the ER, mediates this trafficking. The translocation of TLR7 and TLR9 from the ER to the endosome is also regulated by PRAT4A, which also supports the translocation of TLR4 and TLR1 to the cell surface. A member of ER-resident gp96 functions as a general chaperone for most TLRs including TLR1, TLR2, TLR4, TLR5, TLR7, and TLR9 (not shown here). TLR3 activates the TRIF-dependent pathway to induce type I IFN and inflammatory cytokines in macrophages and cDCs. In pDCs, TLR7 and TLR9 activate NF-κB and IRF7 via MyD88 to induce inflammatory cytokines and type I interferon, respectively. The activation of NF-κB during TLR7 and TLR9 signaling is initiated from the endosome whereas IRF7 activation is initiated from the lysosome-related organelle (LRO) after TLR7 and TLR9 are transported from the endosome to this vesicle in a manner dependent on AP3. MyD88-dependent IRF7 activation in pDCs is mediated by activation of IRAK1, TRAF6, TRAF3, and IKKα and is facilitated by IFN-inducible Viperin expressed in the lipid body. In cDCs and macrophages, TLR7 and TLR9 induce inflammatory responses by activating NF-κB via MyD88 but fail to activate IRF7.

TLR4 is the only TLR that recruits four adaptor proteins and activates two distinct signaling pathways: the "MyD88-dependent" and "TRIF-dependent" pathways (Kawai and Akira, 2010). These two pathways have different kinetics. TLR4 initially recruits TIRAP and MyD88. TIRAP localizes to the plasma membrane via its interaction with PIP2, where it serves to bridge the interaction between MyD88 and TLR4 upon LPS engagement (Barton and Kagan, 2009). MyD88 then recruits IRAKs, TRAF6, and the TAK1 complex, leading to early-phase activation of NF-κB and MAP kinases (Kawai and Akira, 2010). TLR4 is then endocytosed and delivered to intracellular vesicles to form a complex with TRAM and TRIF, which then recruits TRAF3 and the protein kinases TBK1 and IKKi, which catalyze the phosphorylation of IRF3, leading to the expression of type I IFN

(Barton and Kagan, 2009). TRAM-TRIF also recruits TRAF6 and TAK1 to mediate late-phase activation of NF-κB and MAP kinases. The intracellular vesicles, into which TLR4 is delivered, are small GTPase Rab11a-positive recycling endosomes (Husebye et al., 2010). TLR4 accumulates around *Escherichia coli*-containing phagosomes and triggers TRIF-dependent type I IFN induction pathways. Thus, Rab11a specifically regulates TLR4 mobilization from recycling endosomes to phagosomes, a process necessary for TRIF-dependent induction of type I IFN. Whereas activation of the TRIF-dependent pathway is sufficient for type I IFN induction, activation of both the MyD88- and TRIF-dependent pathways is required to drive robust NF-κB and MAP kinase activation and the subsequent induction of inflammatory cytokines. TAG, a splice variant of TRAM, binds intracellular TLR4 to disrupt the TRAM-TRIF interaction, thus terminating activation of the TRIF-dependent pathway (Palsson-McDermott et al., 2009).

TLR2-TLR1 and TLR2-TLR6, which signal through TIRAP and MyD88, are expressed on the cell surface and are recruited to the phagosome during phagocytosis of zymosan or *Staphylococcus aureus* to induce the production of inflammatory cytokines. However, there is some debate about whether TLR2 signaling directly controls phagosome maturation. TLR2 is capable of inducing type I IFN in $CD11c^{-}CD11b^{+}Ly6C^{+}$ inflammatory monocytes infected with vaccinia viruses (Barbalat et al., 2009). The induction of type I IFN by inflammatory monocytes requires TLR2 internalization and is controlled by MyD88-dependent activation of IRF3 and IRF7. Thus, inflammatory monocytes activate unique MyD88-dependent pathways culminating in the activation of IRF3 and IRF7.

A recent report has indicated that TLR2 and TLR4 engagement results in recruitment of mitochondria to macrophage phagosomes and increased production of mitochondrial ROS that has been implicated in mouse macrophage bactericidal activity (West et al., 2011). TRAF6 is translocated to the mitochondria upon bacteria infection, where it interacts with and promotes ubiquitination of ECSIT, resulting in increased mitochondrial and cellular ROS generation. Thus, mitochondria is a platform that regulates TLR-induced bactericidal activity.

TLR7 and TLR9 signal through MyD88, which leads to induction of NF-κB-dependent inflammatory cytokine productions by conventional DCs (cDCs) and macrophages. Notably, TLR7 and TLR9 are exclusively expressed in plasmacytoid DCs (pDCs), which have the capacity to secrete vast amounts of type I IFN rapidly in response to viral infection (Gilliet et al., 2008; Reizis et al., 2011). The production of type I IFN by pDCs after TLR7 and TLR9 engagement is also controlled in a MyD88-dependent fashion. In pDCs, MyD88 forms a complex with TRAF3, TRAF6, IRAK1, IKKα, IRF7, and other proteins such as OPNi and Dock2 (Gotoh et al., 2010; Kawai and Akira, 2006). IRF7 is phosphorylated by IRAK1 and IKKα contained within this complex and translocates into the nucleus to regulate the expression of type I IFN. It should be noted that the intracellular trafficking and retention of the TLR9 signaling complex is thought to determine the induction of inflammatory cytokines or type I IFN. Retention of CpG DNA-TLR9 in the early endosomes boosts MyD88 and IRF7 activation, resulting in IFN production (Honda et al., 2005). A recent report showed that TLR9 activates two signaling pathways within different intracellular compartments (Sasai et al., 2010). TLR9 initially traffics to the early endosomes after CpG DNA stimulation, where it triggers MyD88-TRAF6-dependent NF-κB activation and IL-12p40 production. TLR9 then traffics to the lysosome-related organelles (LRO), where it incorporates TRAF3 to activate IRF7 and induces type I IFN. AP3 specifically controls the trafficking in the latter case and is required to assist TRAF3-IRF7 activation, rather than for TRAF6-NF-κB activation. An IFN-inducing signaling complex (MyD88-IRAK1-TRAF6-IRF7) is formed within lipid bodies by the IFN-inducible Viperin protein (Saitoh et al., 2011). Viperin targets IRAK1 for lysine 63-linked ubiquitination and activates IRF7.

TLR5, which is expressed on the cell surface, signals through MyD88 to induce inflammatory cytokine productions. A recent study suggests that TLR5 in intestinal epithelial cells recruits TRIF, in addition to MyD88, which leads to the activation of NF-κB rather than IRF3 (Choi et al., 2010).

Detection of Bacterial PAMPs by TLRs

Bacteria consist of various PAMPs that are detected by TLRs. Bacterial cell wall components are broadly recognized by cell surface TLRs whereas nucleic acids are recognized by intracellular TLRs. Lipopolysaccharide (LPS) from Gram-negative bacteria is recognized by TLR4 (Akira et al., 2006). TLR2 recognizes a wide variety of PAMPs of both Gram-positive and -negative bacteria and detects lipoproteins and peptidoglycans (PGN) (present in both Gram-positive and Gram-negative bacteria), as well as lipoteichoic acid from Gram-positive bacteria (Akira et al., 2006). TLR2 forms heterodimers with TLR1, TLR6, or other cell surface molecules such as Dectin-1 and CD36 to discriminate between PAMP structures (Akira et al., 2006). The TLR2-TLR1 heterodimer recognizes triacylated lipopeptides from Gram-negative bacteria whereas the TLR2-TLR6 heterodimer recognizes diacylated lipopeptides from Gram-positive bacteria (Akira et al., 2006). The flagellin protein component of the bacterial flagella is recognized by TLR5 (Akira et al., 2006). TLR11 is highly expressed in the kidney and bladder and is implicated in the recognition of uropathogenic bacterial components although a cognate PAMP has not been identified (Akira et al., 2006). Bacterial genomic DNA is recognized by TLR9 (Akira et al., 2006). TLR9 recognizes unmethylated 2′-deoxyribo (cytidine-phosphateguanosine) (CpG) DNA motifs that are frequently present in bacteria and viruses but are rare in mammals. However, it was shown that TLR9 senses the sugar-phosphate backbone of DNA (Haas et al., 2008). TLR9 can recognize vertebrate DNA if it is delivered to the endosomal compartment via a transfection reagent. Thus, the location of DNA rather than specific sequence, modification, or species origin of DNA is important for the recognition by TLR9. Moreover, an interaction between TLR9 and CpG-DNA is facilitated by host proteins such as HMGB proteins and granulin, which are released to the extracellular space by macrophages and DCs (Park et al., 2011; Yanai et al., 2009). These bindings accelerate downstream signaling. Bacterial RNA is also immunostimulatory. TLR7 is reported to recognize RNA from *Group B streptococci* within the lysosomal compartment (Mancuso et al., 2009).

There are redundancies in the recognition of bacterial PAMPs between TLRs and other PRRs (Table 1). NLR members NOD1 (NLRC1), NOD2 (NLRC2), and NALP1 recognize degradation

products of PGN (see review by Elinav et al., 2011, in this issue of *Immunity*). NOD1 and NOD2 induce NF-κB- and MAP kinases-dependent inflammatory responses whereas NALP1 forms an inflammasome to induce the secretion of IL-1β via Caspase-1. Other NLRs, Naip5, and IPAF detect flagellin of intracellular bacteria that is delivered into cytosol through bacterial type IV and type III secretion system and mediate IL-1β secretion (Miao et al., 2007). The NALP3 inflammasomes are activated by many TLR ligands although NALP3 itself may not directly sense PAMPs. AIM2, which is not a member of NLR but forms an inflammsome along with ASC and Caspase-1, senses dsDNA in the cytoplasm and mediates innate immune responses to *Francisella tularensis* and *Listeria monocytogenes* (Fernandes-Alnemri et al., 2010; Rathinam et al., 2010).

Impact of TLRs on Innate Immune Responses to Bacteria

The role of TLRs in in vivo bacterial infections has been studied in mice deficient in individual TLRs. *Salmonella typhimurium*, a Gram-negative bacterium that can replicate within macrophages, has at least four PAMPs detected by TLRs: lipoprotein (TLR2), LPS (TLR4), flagellin (TLR5), and CpG-DNA (TLR9) (Gerold et al., 2007). TLR4-deficient mice challenged with *S. typhimurium* are more susceptible to infection than control mice and show increased bacterial accumulation within the mesenteric lymph nodes (Weiss et al., 2004). TLR2-TLR4 double-deficient mice are more susceptible than TLR4 single-knockout mice, although TLR2 deficiency does not impair survival (Weiss et al., 2004), suggesting that TLR2-mediated responses are functional in the absence of TLR4. However, TLR2-TLR4 double-deficient mice survive when infected with low doses of bacteria, which may reflect natural rates of infection. TLR5 also plays a protective role subsequent to intraperitoneal infection with *S. typhimurium*, intranasal infection with *Pseudomonas aeruginosa*, and urinary tract infection with *E. coli* (Andersen-Nissen et al., 2007; Feuillet et al., 2006). However, TLR5 is deleterious in hosts orally infected with *S. typhimurium*. TLR5-deficient mice show increased survival resulting from decreased migration of bacteria from the intestinal tract to the mesenteric lymph nodes (Uematsu et al., 2006). Thus, TLR5 can be either beneficial or detrimental to the host, depending on the bacterial dose and route of infection. Recently, it was shown that TLR2-TLR4 double-knockout mice are highly susceptible to oral infection with *S. typhimurium*, whereas TLR2-TLR4-TLR9 triple-knockout mice, which have a more severe phenotype in terms of cytokine induction than TLR2-TLR4 double-knockout mice, are nevertheless less susceptible to infection (Arpaia et al., 2011). In the absence of these three TLRs, bacteria fail to upregulate the *Salmonella* pathogenicity island 2 (SPI-2) genes that encode the proteins that are required for survival within the *Salmonella*-containing vacuole (SCV) and are, therefore, unable to replicate within macrophages. TLR signaling leads to the acidification of the SCV, which is required for SPI-2 induction. Thus, *S. typhimurium* may use TLRs as signals that support the expression of their own virulence genes, which are required for growth and systemic infection.

S. aureus is a Gram-positive bacterium that contains several TLR PAMPs. The TLR2-TLR6 heterodimer recognizes lipoprotein and lipoteichoic acid. TLR2-deficient mice show an increased bacterial burden and succumb after systemic infection with *S. aureus* (Takeuchi et al., 2000). Moreover, they also show an increased bacterial burden and disease severity after nasal, cutaneous, and corneal infection (Gerold et al., 2007), suggesting that TLR2-mediated recognition of lipoproteins induces a protective response to *S. aureus*. In contrast, in a brain abscess model, there was no difference on bacterial burden, survival, and neutrophil infiltration between TLR2-deficient and control mice (Miller et al., 2006). MyD88-deficient mice are more susceptible to infection than TLR2-deficient mice in various infection models (Miller et al., 2006; Takeuchi et al., 2000), suggesting a contribution by other TLRs and/or members of IL-1 receptor family. Indeed, IL-1 receptor-deficient mice are protected against *S. aureus*-induced sepsis and show reduced cytokine production and impaired neutrophil recruitment in cutaneous infection models (Verdrengh et al., 2004). Therefore, both IL-1 receptor and TLR2 signaling are coupled to induce protective immune responses against *S. aureus* infection.

Cooperation between TLRs and Other PRRs in the Regulation of Innate and Adaptive Immune Responses to Mycobacterium Infection

Tuberculosis can be acquired by inhalation of *Mycobacterium tuberculosis* (Mtb) in aerosols and dust. Mtb is initially internalized into the phagosomes of alveolar macrophages (AM), where they multiply. Initial infection by Mtb is probably sensed by TLR2, TLR4, and TLR9, upregulating the transcription of inflammatory cytokines such as IL-1β, IL-12, TNF-α, and IL-6, which control the infection (Gerold et al., 2007; Saiga et al., 2011). MyD88-deficient mice are extremely susceptible to Mtb infections associated with increased bacterial burden, with a concomitant reduction of IFN-γ, IL-12, TNF-α, and NOS2 production (Saiga et al., 2011). This suggests that TLR-mediated recognition of Mtb is critical for innate responses to Mtb. However, there are conflicting results regarding the in vivo importance of individual TLRs in protective responses to Mtb. Some reports indicate that mice deficient for TLR2, TLR4, or TLR9 exhibit no, or only minor, susceptibility to low-dose Mtb infection whereas others suggest that TLR2- and TLR9-deficient mice are highly susceptible to infection by high-dose Mtb (Saiga et al., 2011). Moreover, TLR4-deficient mice show increased susceptibility (Saiga et al., 2011). However, a study with TLR2-TLR4-TLR9 triple-knockout mice infected with Mtb found controlled replication of Mtb in the lung, similar to that observed in control mice (Hölscher et al., 2008). Thus, the role of individual TLRs that recognize Mtb PAMPs may be largely redundant in mediating protective immune responses to low-dose aerosol Mtb infection. In addition to the innate immune responses that are critical for the initial defense against Mtb infection, the adaptive immune response also has an important role to play against chronic infections. Antigen-specific Th cells that produce IFN-γ enhance the killing activity of infected macrophages. Notably, although MyD88-deficient mice are highly susceptible to infection and exhibit an increased bacterial burden, the induction of Th1 cell development as well as macrophage effecter responses are unimpaired (Saiga et al., 2011). Thus, induction of adaptive immune responses to Mtb may be mediated by PRRs other than TLRs. Alternatively, other PRRs may compensate for TLR-dependent Th1 cell development.

In addition to TLRs, NLRs and CLRs are involved in the recognition of Mtb. Impaired cytokine induction by macrophages and DCs after infection by Mtb is observed in mice deficient for NOD2 (Saiga et al., 2011). NOD2 acts synergistically with TLR2 to induce inflammatory cytokine production after Mtb infection. However, NOD2 single- and NOD2-TLR2 double-knockout mice are able to control Mtb replication and are only as susceptible to virulent Mtb infection as control mice (Gandotra et al., 2007). Mice deficient in IL-1β or IL-1 receptor have high mortality (similar to MyD88-deficient mice) (Fremond et al., 2007), which suggests that IL-1 receptor signaling plays a dominant role in determining the phenotype of MyD88-deficient mice. Notably, IL-1β levels in the lungs of Mtb-infected mice are increased in MyD88-, Caspase-1-, and ASC-deficient mice, and Caspase-1- and ASC-deficient mice are less susceptible to Mtb infection than IL-1β-deficient mice (Mayer-Barber et al., 2010; McElvania Tekippe et al., 2010). Furthermore, dispensable role for Caspase-1 and NALP3 is also reported although ASC is partially involved (McElvania Tekippe et al., 2010). These findings suggest that there are as yet uncharacterized innate pathways that control IL-1β release during Mtb infection.

Certain CLRs recognize mycobacterial components and induce inflammatory cytokines. These include the mannose receptor, DC-SIGN, Dectin-1, and Mincle, although their cognate PAMPs have not yet been identified (Geijtenbeek and Gringhuis, 2009; Marakalala et al., 2010). The requirement for these CLRs in the control of Mtb infections in vivo varies between various studies, principally because of differences in the cell types or mycobacterial species used. However, it appears that CARD9 plays a critical role. CARD9 was originally identified as an essential molecule involved in antifungal immunity (Gross et al., 2006). Subsequent studies show that CARD9 is also involved in signaling through multiple PRRs, including Dectin-1, TLRs, NOD2, and RIG-I (Mócsai et al., 2010; Poeck et al., 2010). Thus, CARD9 may converge the signaling from multiple PRRs that mediate recognition of Mtb. CARD9-deficient macrophages and DCs show reduced production of inflammatory cytokines after infection with Mtb, indicating that CARD9 mediates innate immune responses to Mtb. CARD9-deficient mice challenged with virulent Mtb rapidly succumb to systemic inflammatory diseases associated with augmented cell death in the lung and enhanced recruitment of granulocytes. Failure to suppress excessive inflammation in the absence of CARD9 is most probably due to reduced production of IL-10 (Dorhoi et al., 2010). Thus, CARD9 signaling is critical for the control of Mtb infection. However, it is notable that CARD9 deficiency does not affect T cell responses to Mtb (Dorhoi et al., 2010).

Recognition of Viral Nucleic Acids through TLRs

Viral nucleic acids act as PAMPs and are recognized by multiple TLRs. TLR3, TLR7, TLR8, and TLR9 are involved in the recognition of viral nucleotides such as double-stranded RNA (dsRNA) (TLR3), single-stranded RNA (ssRNA) (TLR7-TLR8), and DNA (TLR9) (Blasius and Beutler, 2010). The hallmark of these nucleic acid-sensing TLRs is that they potently promote the production of type I IFN, in addition to the other inflammatory cytokines that are induced by all TLRs.

TLR3 was originally identified to recognize a synthetic analog of dsRNA polyinosinic-polycytidylic acid (poly(I:C)). TLR3 also recognizes genomic RNA from dsRNA viruses (including reoviruses) and dsRNA produced during replication of ssRNA viruses (such as influenza A virus [IAV], encephalomyocarditis virus [EMCV], and West Nile virus [WNV]) and dsDNA viruses (herpes simplex virus 1 [HSV-1] and murine cytomegarovirus [MCMV]) (Akira et al., 2006; Blasius and Beutler, 2010).

TLR7 and human TLR8 mediate the recognition of ssRNA derived from ssRNA viruses and synthetic antiviral imidazoquinoline components (Akira et al., 2006; Blasius and Beutler, 2010). TLR7 recognizes ssRNA derived from HIV or influenza virus and synthetic polyuridine ssRNA. TLR7 and TLR9 are both highly expressed by pDCs (Akira et al., 2006; Blasius and Beutler, 2010; Gilliet et al., 2008; Reizis et al., 2011). Induction of type I IFN by pDCs occurs independently of the replication of enveloped viruses such as IAV. TLR7 is likely to engage viral RNA within endosomal compartments that are delivered subsequent to virus uptake (Blasius and Beutler, 2010). Moreover, pDCs can recognize replicating viruses, such as VSV, that enter the cytoplasm. The delivery of cytosolic viral RNA to the lysosome via autophagy, a process involving lysosomal degradation of cellular organelles or pathogens, results in TLR7-mediated recognition and activation of signaling (Lee et al., 2007). TLR8 is expressed in various tissues, with the highest expression in monocytes.

TLR9 recognizes the viral DNA of DNA viruses that contain sequences rich in CpG-DNA motifs. TLR9 is involved in recognition of HSV-1, HSV-2, and MCMV (Akira et al., 2006; Blasius and Beutler, 2010; Gilliet et al., 2008; Reizis et al., 2011). Type I IFN production by pDCs after infection by these viruses is completely dependent upon TLR9, suggesting that TLR9 serves as a sensor for DNA viruses in pDCs.

Sensing of Viral Infections by TLR2, TLR4, and TLR13

Cell-surface TLRs such as TLR2 and TLR4 recognize viral products. TLR4 recognizes the fusion protein from RSV and induces the production of inflammatory cytokines (Akira et al., 2006). TLR4-deficient mice show reduced viral clearance, accompanied by low levels of infiltrating mononuclear cells and reduced production of IL-12. The envelope protein of mouse mammary tumor virus (MMTV) is also recognized by TLR4 (Akira et al., 2006). MMTV enhances the expression of its entry receptor to facilitate viral entry via TLR4. Thus, MMTV may subvert host antiviral responses via its interaction with TLR4. TLR4-deficient mice are protected from acute lung injury caused by inactivated H5N1 avian influenza virus. TLR4-mediated sensing of oxidative stress is considered to contribute to inflammation (Imai et al., 2008). TLR2 senses viral components, such as measles virus hemagglutinin protein, human CMV, and HSV-1, and induces inflammatory cytokines (Akira et al., 2006). In addition, TLR2 is capable of inducing type I IFN in a cell type-specific manner. When stimulated with inactivated vaccinia virus, inflammatory monocytes produce type I IFN via TLR2, which is in contrast to macrophages and other types of DCs where TLR2 induces inflammatory cytokines, but not type I IFN (Barbalat et al., 2009). Moreover, induction of TLR2-dependent type I IFN does not require nucleic acids. TLR13 is localized intracellularly and interacts with UNC93B1 (Shi et al., 2011). TLR13 is implicated in the recognition of vesicular stomatitis virus (VSV) for the induction of type I IFN.

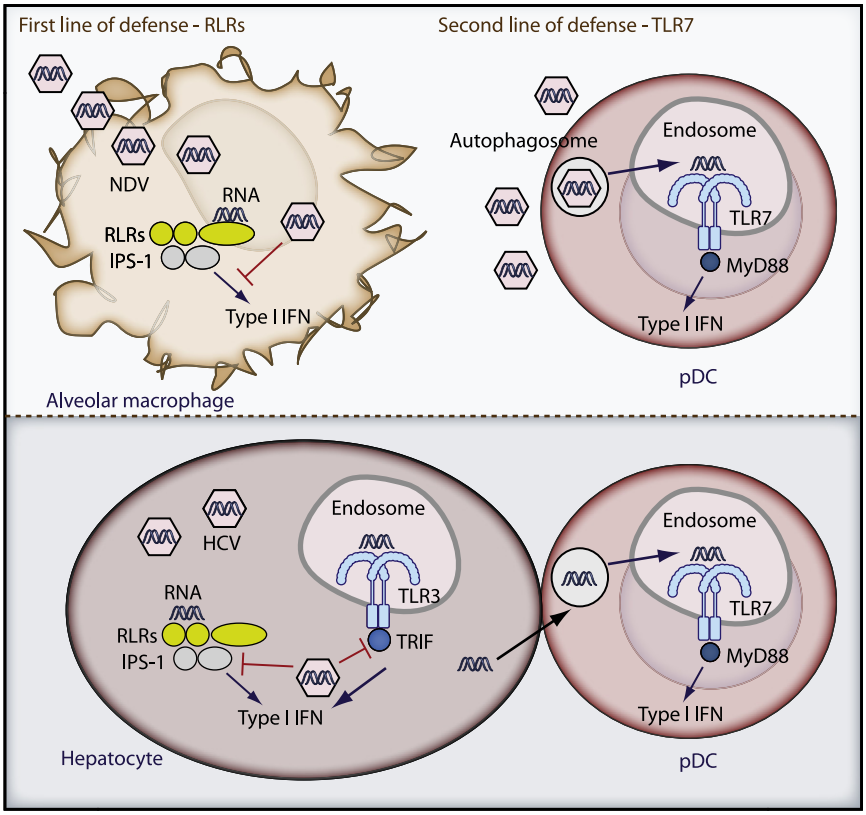

Figure 2. Interplay between TLRs and RLRs during Virus Infection

AM recognize RNA viruses such as NDV via RLRs. pDCs are able to produce type I IFN when the first line of RLR-mediated defensive responses in AM is suppressed or disrupted by the virus (top). HCV is recognized by TLR3 and RIG-I in hepatocytes. However, HCV evades type I IFN responses by expressing viral NS3-NS4A protease, which cleaves TRIF and IPS-1. HCV RNA is delivered from infected hepatocytes to the pDCs that are migrated into the liver upon direct cell-to-cell contact, which triggers type I IFN production via TLR7. The RNA may be delivered through autophagosomes or exosomes (bottom).

Role of TLR3 in Control of Virus Infection

Although TLR3 triggers the production of type I IFN and inflammatory cytokines required for eliminating viruses, the in vivo requirement for TLR3 for protection against infection is controversial. TLR3-deficient mice are more susceptible than control mice to MCMV infections, probably because of reduced production of type I IFN, IL-12p40, and IFN-γ and a reduction in NK and NKT cell activation (Tabeta et al., 2004). TLR3-mediated IFN-γ but not type I IFN signaling is necessary for protection against infection by coxsackievirus group B serotype 3 (Negishi et al., 2008). Also, TLR3-deficient mice are more susceptible to infection with virulent WNV (Daffis et al., 2008). Early viral entry into the CNS and the virus titer in the peripheral tissues are both increased in TLR3-deficient mice. These findings indicate that TLR3-mediated RNA recognition promotes protective immunity. However, there are reports indicating that TLR3 contributes to pathogenesis rather than protection. TLR3-deficient mice show increased survival rates after WNV infection (Wang et al., 2004). The production of inflammatory cytokines in the periphery is also decreased in TLR3-deficient mice, resulting in inhibition of viral entry into the brain because of decreased blood-brain barrier permeability. TLR3-deficient mice infected with IVA also exhibit increased survival despite the higher viral loads in the lung (Le Goffic et al., 2006). The increased survival is probably due to the abrogation of the IVA-induced overproduction of cytokines, which is detrimental to the host. Moreover, it has been reported that TLR3 is not necessary for antiviral responses because TLR3 deficiency does not affect survival or $CD4^+$ T and $CD8^+$ T cell responses after infection with MCMV, reoviruses, lymphocytic choriomeningitis virus, and VSV (Edelmann et al., 2004).

Interplay between TLRs and Other PRRs during Viral Infection

Infection by RNA viruses is sensed by TLR7 and RLRs, both of which induce type I IFN. Ex vivo experiments indicate that type I IFN production by pDCs subsequent to RNA viruses such as IAV, VSV, and Newcastle disease virus (NDV) relies on TLR7, whereas production by cDCs, macrophages, and fibroblasts depends on RIG-I (Akira et al., 2006). Interplay between TLRs and RLRs in different cell types during viral infection plays an important role in antiviral responses. When mice were intranasally infected with NDV, pDCs were not the major source of type I IFN. Rather, AM and cDCs produced type I IFN via RLRs (Kumagai et al., 2007). Interestingly, pDCs are capable of producing type I IFN in the absence of functional AM, suggesting that pDCs play a dominant role when the first line of AM-mediated defensive responses is disrupted (Kumagai et al., 2007). Given that many viruses have evasion mechanisms that suppress RLR signaling (i.e., NS1 of IAV suppresses RIG-I signaling), pDCs may function as a backup for antiviral responses when RLR signaling is suppressed by viruses (Figure 2).

Hepatitis C virus (HCV) is recognized by TLR3 and RIG-I. However, HCV evades type I IFN responses by expressing viral NS3-NS4A protease, which cleaves TRIF and IPS-1 (Horner and Gale, 2009; Lemon, 2010; Rehermann, 2009). Thus, type I IFN production in infected hepatocytes is suppressed. However, HCV induces robust type I IFN production by pDCs, which infiltrate the liver during infection. Production of type I IFN by pDCs in the liver is dependent on direct cell-to-cell contact with infected hepatocytes but is independent of the uptake of viral particles by the pDCs (Figure 2; Takahashi et al., 2010). Thus, viral RNA in the infected hepatocytes is likely to trigger type I IFN production by pDCs. When a replication-defective RNA is transfected into pDCs, type I IFN is induced in TLR7-dependent manner. Collectively, HCV RNA is delivered from infected hepatocytes to the pDCs upon direct cell-to-cell contact, which

triggers type I IFN production via TLR7. It may be possible that the RNA is transported via vesicle transfer through autophagosomes or exosomes. It is reported that the ability of pDCs from HCV-infected patients to induce type I IFN is normal although their number of pDCs is reduced (Kanto et al., 2004; Shiina and Rehermann, 2008). A greater understanding of the mechanisms underlying the function of liver pDCs in the regulation of HCV infection is required.

Recognition of Fungal PAMPs by TLRs

Invasive *Candida* infection is life threatening in immunocompromised patients. The innate immune recognition of *Candida* infection by DCs induces the production of an array of inflammatory cytokines and the upregulation of costimulatory molecules that support the differentiation of naive T cells into Th1, Th2, Th17, and Treg cells. In a mouse model of *Candida albicans* infection, Th1 and Th17 cell responses are crucial for protection against infection whereas Th2 and Treg cell responses suppress innate immune responses that are deleterious to the host. *Candida* spp. contains multiple PAMPs such as β-glucan, chitin, mannan, proteins, and nucleic acids, which are recognized by at least five TLRs (TLR2, TLR4, TLR6, TLR7, and TLR9) as well as CLRs and NLRs (Netea and Maródi, 2010; Romani, 2011).

A general role of TLR- and/or IL-1 receptor family-mediated responses in host defense against *C. albicans* is reported in studies on MyD88-deficient mice that are highly susceptible to infection (Bellocchio et al., 2004). However, there are conflicting reports regarding the role of individual TLRs in the host innate and adaptive immune responses to *Candida* spp. Phospholipomannan from *C. albicans* stimulates DCs and macrophages to produce TNF-α, IL-1β, and IL-10 via TLR2 (Netea and Maródi, 2010), leading to the generation of Th2 and Treg cells. TLR2-deficient mice are more resistant to lethal disseminated candidiasis than control mice, and this is accompanied by decreased production of IL-10 and increased production of IFN-γ and IL-12 (Netea et al., 2004; Sutmuller et al., 2006). Thus, TLR2-mediated recognition of *Candida* spp. may be rather deleterious to the host. However, other studies suggest protective or dispensable role of TLR2 in antifungal responses in vivo (Netea and Maródi, 2010). TLR2-deficient mice produce lower levels of TNF-α and chemokines and are more susceptible to disseminated candidiasis than control mice. Moreover, TLR2-deficient mice are not protected against a second challenge with *C. albicans* hyphae after sublethal infection with virulent *C. albicans* (Bellocchio et al., 2004). Although TLR2-deficient mice show lower levels of antibody production than control mice after challenge with low-virulence *Candida* spp., like the control mice they are protected against rechallenge of virulent *C. albicans* (Netea and Maródi, 2010). The TLR6-TLR2 heterodimer recognizes zymosan, and TLR6 deficiency modestly impairs cytokine production after *C. albicans* infection. However, TLR6-deficient mice do not show increased susceptibility (Netea and Maródi, 2010).

TLR4 recognizes mannans expressed by *Saccharomyces cerevisiae* and *C. albicans*. Short, linear O-linked mannans are recognized by TLR4, resulting in the production of cytokines such as TNF-α (Netea and Maródi, 2010). The role of TLR4 in host defense against *C. albicans* is complicated. It was reported that TLR4-defective C3H/HeJ mice were more susceptible to *C. albicans* infection than control mice, and this was associated with decreased production of the chemokines by macrophages and impaired recruitment of neutrophils (Netea and Maródi, 2010). Furthermore, TLR4-deficient mice are susceptible to reinfection with *Candida* hyphae after priming with low-virulence *Candida* yeast (Netea and Maródi, 2010). By contrast, other studies indicate that TLR4 deficiency does not influence susceptibility to *Candida* yeast and that TLR4-deficient mice survive longer than control mice when systemically infected with *Candida* hyphae (Netea and Maródi, 2010).

In addition to cell-surface TLRs, intracellular TLRs such as TLR7 and TLR9 participate in the recognition of fungal nucleic acids that are released into TLR-containing vesicles during digestion by phagocytes. Fungal DNA induces the production of inflammatory cytokines by DCs via TLR9 (Netea and Maródi, 2010; Romani, 2011). $CD4^+$ T cells from TLR9-deficient mice show higher levels of IL-4 and lower levels of IFN-γ production after challenge with *C. albicans* than those from control mice, suggesting that the TLR9-dependent pathway skews T cells toward Th1 cell polarization. However, TLR9-deficient mice are protected against rechallenge of virulent *C. albicans* (Bellocchio et al., 2004). A protective role for TLR9 is suggested by a study with a mouse model of vaccination. Vaccination of mice with *Aspergillus* proteins plus a TLR9 agonist enhances Th1 cell responses and protection against aspergillosis (Bozza et al., 2002).

Challenging cDCs with *Candida* spp. triggers type I IFN release, which is abrogated by TLR7 deficiency (Bourgeois et al., 2011). This induction requires phagocytosis of *Candida* spp. Thus, *Candida* spp. RNA is likely to be sensed by TLR7. The *Candida* burden is decreased in mice lacking type I IFN receptor (IFNAR). A significant increase in the incidence of splenomegaly is observed after *Candida* infection in IFNAR-deficient mice. Therefore, *Candida* infection-induced type I IFN production by cDCs may suppress the inflammatory responses that are required for host defensive responses. By contrast, another study shows that mice lacking type I IFN signaling challenged intravenously with *C. albicans* die earlier because of a failure of controlling replication, suggesting that type I IFN production by cDCs is protective in this case (Biondo et al., 2011). Collectively, it appears that individual TLRs elicit different but overlapping immune responses against *Candida* infection.

Collaboration between TLRs and Other PRRs in the Regulation of Antifungal Immunity

Certain TLRs cooperate with members of CLRs to mount antifungal immunity (Netea and Maródi, 2010; Romani, 2011). Dectin-1 recognizes β-1,3-linked glucan and mediates phagocytosis of fungus by DCs. Dectin-1 triggers signaling pathways involving the Syk tytosine kinase and CARD9, which leads to the production of IL-2, IL-10, and other cytokines. This pathway drives Th17 cell responses and is required for protection against *Candida* infection in vivo although difference of *C. albicans* strains or knockout background may influence a requirement of this pathway in host defense (Netea and Maródi, 2010; Romani, 2011). The importance of CARD9 against *C. albicans* infection in vivo is also shown in humans and mice (Gross et al., 2006; Glocker et al., 2009). Notably, dectin-1 collaborates with TLR2

or TLR4 to trigger cytokine production upon recognition of *C. albicans* and zymosan (Goodridge and Underhill, 2008). Galectin 3 and Mannose receptor are involved in supporting TLR2-mediated innate and Th cell responses to *C. albicans* (Netea and Maródi, 2010; Romani, 2011). In addition to CLRs, NALP3 is also involved in antifungal responses. The NALP3 inflammasome is activated by infection by *C. albicans* and *S. cereviciae*, and the activation of Caspase-1 and the production of IL-1β triggered by these fungi are dependent on NALP3 (Gross et al., 2009; Hise et al., 2009; Joly et al., 2009; Kumar et al., 2009). IL-1β is thought to mediate neutrophil recruitment and the generation of ROS, and IL-1 receptor-deficient mice consistently show increased susceptibility to disseminated candidiasis. NALP3-deficient mice are more susceptible to *Candida* infections than control mice. Fungal β-glucans are responsible for NALP3 inflammasome-dependent IL-1β release, which requires phagocytosis and Syk activation. Thus, IL-1β production by β-glucans may be regulated by two steps: one involving the dectin-1-dependent induction of pro-IL-1β expression and the other involving NALP3-mediated processing of pro-IL-1β to mature IL-1β. Moreover, it was shown that ATP drives the NALP3 inflammasome activation in response to zymosan (Lamkanfi et al., 2009).

Recognition of Protozoa and Parasites by TLRs

Components of protozoan parasites such as *Trypanosoma cruzi*, *Trypanosoma brucei*, *Toxoplasma gondii*, *Leishmania major*, and *Plasmodium falciparum* are sensed by TLRs (Gazzinelli and Denkers, 2006). *T. cruzi*-derived glycosylphosphatidylinositol (GPI) anchors activate macrophages to induce inflammatory cytokines through TLR2 (Gazzinelli and Denkers, 2006). In addition, free GPI anchors (glycoinositolphospholipids containing ceramide) derived from *T. cruzi* trigger the production of chemokines via TLR4 (Gazzinelli and Denkers, 2006). Similarly, TLR2 and/or TLR4 are involved in the recognition of GPI anchors from *T. gondii*, *L. major*, and *P. falciparum* (Gazzinelli and Denkers, 2006). In addition to GPI anchors, structural proteins and genomic DNA are also recognized by TLRs (Gazzinelli and Denkers, 2006). The *T. cruzi*-derived protein Tc52, which is related to the thiol-disulfide oxidoreductase family, triggers inflammatory cytokine induction by DCs via TLR2. The soluble fraction of *T. gondii* tachyzoites contains a potent inducer for IL-12 known as soluble Toxoplasma antigen. This is mediated by a profilin-like molecule (a small ubiquitous protein with a known actin-binding protein that is implicated in parasite motility and invasion), which is recognized by mouse TLR11 (Yarovinsky et al., 2005). Human TLR11 is, however, nonfunctional because of the presence of a stop codon in the gene. Notably, TLR9 is involved in the recognition of the heat-labile non-DNA fractions and hemozoin derived from *P. falciparum* (Coban et al., 2005). Hemozoin is an insoluble crystal generated as a by-product of the detoxification process after parasitic digestion of host hemoglobin and induces the production of inflammatory cytokines and type I IFN by DCs via TLR9. TLR9-deficient mice display partial resistance to lethal infection by the rodent malaria parasite *Plasmodium yoelii* resulting from impaired Treg cell activation (Hisaeda et al., 2008). Thus, TLR9 may be targeted by malaria parasites for evasion.

Role of TLRs in Protection against Protozoa and Parasites

Mice deficient for MyD88 are highly susceptible to infection with *L. major*, *T. brucei*, *T. cruzi*, *Plasmodium berghei*, and *T. gondii*, and this is associated with decreased production of the Th1 cytokines IFN-γ and IL-12 (Gazzinelli and Denkers, 2006). This indicates that TLR-mediated recognition of protozoan parasites is critical for immediate activation of the innate immune system and the consequent induction of Th1 cells. Mice with a deficiency of MyD88 specifically in DCs show defective IL-12 production and compromised Th1 cell responses after *T. gondii* infection (Hou et al., 2011). Furthermore, MyD88 signaling in DCs, but not neutrophils or macrophages, is required for host defense during acute infection by *T. gondii* before Th1 cell responses can be initiated. Thus, MyD88 signaling in DCs is critical for IL-12-dependent innate and adaptive immune responses to *T. gondii* in vivo.

However, in contrast to a critical role of MyD88 in survival, inactivation of TLR2, TLR4, or TLR9 has no (or only a modest) effect on survival or immune responses during infection with *L. major*, *T. brucei*, *T. cruzi*, *P. berghei, P. falciparum*, and *T. gondii*, suggesting that multiple TLRs orchestrate the protective responses (Adachi et al., 2001; Bafica et al., 2006; Coban et al., 2007; Kropf et al., 2004; Mun et al., 2003; Ropert and Gazzinelli, 2004). Indeed, TLR2 and TLR9 double deficiency in mice results in increased susceptibility to *T. cruzi* infection, which is comparable to MyD88-deficient mice. TLR11-deficient mice are more susceptible to *T. gondii* infection, with a concomitant decrease in IL-12 and IFN-γ production; however, they are not as susceptible as MyD88-deficient mice (Bafica et al., 2006), suggesting a partial involvement of TLR11 in mounting protective Th1 cell responses. It is reported that pDCs from mice infected with *T. gondii* are able to produce IL-12 and present antigen to prime naive $CD4^+$ T cells through TLR11 (Pifer et al., 2011), suggesting an unexpected role for pDCs in the development of adaptive immune responses to nonviral pathogens.

Recently, it was shown that mice with a loss of functional UNC93B1, which fail to respond to TLR3, TLR7, or TLR9 agonists, show decreased IL-12 production by DCs and are highly susceptible to infection with *T. gondii* although deficiency in TLR3, TLR7, or TLR9 alone does not influence susceptibility (Melo et al., 2010; Pifer et al., 2011). This suggests a combined role for nucleic acid-sensing TLRs in host resistance to *T. gondii*. Notably, TLR11 is found to be expressed in the ER along with UNC93B1. Thus, UNC93B1 is likely to regulate not only nucleic acid-sensing TLRs but also protein-sensing TLR11 in intracellular compartments to establish antiparasitic immunity.

Role of TLRs in Shaping Adaptive Immune Responses

The activation of innate immunity is essential for instructing antigen-specific adaptive immune responses. Indeed, TLR ligands such as monophosphoryl lipid A (TLR4 ligand), CpG-DNA (TLR9 ligand), and imidazoquinolines (TLR7 ligand) are currently developing for use as vaccine adjuvant against infectious diseases, allergy, and tumors. TLRs are mainly expressed in antigen-presenting cells such as DCs, macrophages, and B cells. Careful analyses demonstrated that individual TLRs are

expressed by distinct DC subsets. TLR3 is strongly expressed by CD8α^+ DCs in mice and CD141 (BDCA3)$^+$ DNGR-1 (CLEC9A)$^+$ DCs in humans which have high phagocytic activity (Edwards et al., 2003; Villadangos and Shortman, 2010). These cells mount efficient CD8$^+$ T cell responses when they engulf either dsRNA-loaded cells or virally infected cells and trigger DC maturation and the presentation of viral antigen on MHC class I molecules, both of which stimulate CD8$^+$ T cell responses (Schulz et al., 2005). This process, referred to as cross-priming, is abrogated by TLR3 deficiency, indicating a critical role of TLR3 in mounting virus-specific CD8$^+$ T cell responses. TLR5 is highly expressed in CD11c$^+$CD11b$^+$ lamina propria DCs in the small intestine, which have properties to promote Th1 and Th17 cell differentiation as well as the differentiation of naive B cells into IgA-producing plasma cells in response to flagellin (Uematsu et al., 2008). pDCs selectively express TLR7 and TLR9 to recognize viral nucleic acids. TLR7-mediated type I IFN production by pDCs is essential for both Th1 cell polarization and antibody production when mice are given inactivated whole-influenza vaccine (Koyama et al., 2010). Thus, TLR signaling in DCs can instruct adaptive immune responses both ex vivo and in vivo.

However, TLRs and other PRRs often share the same adjuvant for recognition and activation of immune responses. These include poly(I:C) (recognized by TLR3 and MDA5), flagellin (recognized by TLR5 and IPAF/Naip5), and hemozoin (recognized by TLR9 and activates the NALP3 inflammasome). When poly(I:C) was given along with antigen into mice, antigen-specific antibody production and the differentiation of CD4$^+$ and CD8$^+$ T cells were both abrogated in mice lacking both TRIF and IPS-1 (Kumar et al., 2008). Furthermore, the recognition of poly(I:C) by MDA5 expressed by DCs, monocytes, and stromal cells and the induction of type I IFN are required to support Th1 cell responses (Longhi et al., 2009). Together, the interplay between TLR3 and RLRs is required for robust adaptive immune responses induced by poly(I:C).

The ability of flagellin to promote innate and adaptive immune responses was studied with mice deficient for TLR5, IPAF, or both (Vijay-Kumar et al., 2010). The production of CXCL1 after in vivo injection of flagellin is totally dependent on TLR5 whereas the production of IL-18, which is generated by the inflammasome, is regulated by IPAF. However, TLR5 or IPAF deficiency did not abrogate ability of mice to generate antigen-specific antibodies whereas TLR5 and IPAF double-deficient mice showed reduced antibody titer, indicating that both TLR5- and IPAF-mediated flagellin recognitions are required in the adjuvanticity. Notably, MyD88-deficient mice that are unresponsive to various TLR ligands and IL-1β and IL-18 generated by the inflammasome are capable of mounting antibody responses although the titer is slightly reduced. These findings suggest that signaling components other than MyD88 in TLR5 and IL-1 receptor family members contribute to the regulation of humoral responses. A recent report has implicated that expansion of flagellin-specific CD4$^+$ T cells requires the expression of TLR5 by DCs (Letran et al., 2011). However, MyD88 was unexpectedly dispensable for the generation of CD4$^+$ T cells. Thus, TLR5 may function as endocytoic receptor for flagellin in DCs that enhance presentation of flagellin-derived peptide to CD4$^+$ T cells through MyD88-independent pathway.

In a vaccination model, the adjuvant effects of synthetic hemozoin such as antigen-specific antibody production are independent on TLR9, but totally dependent on MyD88, suggesting the involvement of other TLRs and/or IL-1 receptors in shaping adaptive immunity (Coban et al., 2010). Moreover, it is possible that the NALP3 inflammasome is also involved, because hemozoin is able to activate the NALP3 inflammasome to induce secretion of IL-1β, which eventually activates MyD88-dependent signaling pathways (Dostert et al., 2009; Griffith et al., 2009; Shio et al., 2009).

The essential role of TLR7 in shaping adaptive immunity against IAV infection has been studied. In a mouse model of IAV infection, Th1 cell responses and the production of virus-specific total IgG, IgG2a, and IgG2c were reduced in TLR7- and MyD88-deficient mice, while that of IgG1 was increased (Koyama et al., 2007). Moreover, TLR7 is critical for Th1 cell polarization and antibody production when mice are given inactivated whole-influenza vaccine (Koyama et al., 2010). Thus, both Th1 cell responses and antibody production are regulated by TLR7 in vivo. It is notable that CTL induction occurs independently via TLR7 and RLRs (Koyama et al., 2007). Multiple types of innate cells in the lung are implicated in mediating the generation of CTLs, although the innate immune pathways that control CTL activation in these cells remain uncharacterized. IAV induces IL-1β release through NALP3 inflammasome (Ichinohe, 2010), suggesting the possibility that NALP3 participates in this pathway. In contrast to studies indicating a protective role for TLR7, others report that TLR7 is not necessary in shaping antibody responses (Seo et al., 2010). The differences between these studies may be due to the different experimental conditions used, such as virus strains and infection protocol.

In contrast, several adjuvants do not contain TLR agonists but do effectively induce antigen-specific adaptive immune responses. For example, humoral and Th1 cell responses induced by DNA vaccines, which have antigenic sequences as well as elements that enhance innate immune response, do not require TLR9, RLRs, or DAI but do require STING and TBK1 (Ishii et al., 2008; Barber, 2011), suggesting that unidentified DNA sensors that signal through STING and TBK1 may play critical role. Adjuvant effects of alum such as Th2 cell-biased humoral responses are independent on TLRs but are regulated by the NALP3 inflammasome (Spreafico et al., 2010). However, the contribution of the NALP3 inflammasome in the adjuvanticity of alum is controversial (Spreafico et al., 2010). Mycobacterial glycolipid PAMP treharose-6,6-dimycolate (TDM, also known as cord factor) is used as an adjuvant and is recognized by Mincle. Inflammatory cytokine production and Th1 and Th17 cell responses to TDM are reduced in Mincle-deficient mice (Ishikawa et al., 2009; Schoenen et al., 2010). CARD9 and Syk are also involved in this pathway. Thus, the Mincle-Syk-CARD9-dependent pathway is critical in initiating innate and adaptive immunity triggered by mycobacterial glycolipid (Werninghaus et al., 2009). Conventional adjuvants such as Freund's complete adjuvant, Freund's incomplete adjuvant, and monophosphoryl-lipid A/trehalose dicorynomycolate adjuvant drive effective antibody responses in the absence of TLR signaling even though they contain TLR ligands (Gavin et al., 2006), suggesting dispensable role of TLRs in induction of adaptive immunity.

Mutations of Genes Encoding TLRs and Their Signaling Molecules in Human Immunodeficiency

After the discovery of TLRs, much progress has been made in our understanding that the mutations of genes involved in the regulation of innate immunity is linked to primary immunodeficiencies (PIDs) in humans (Casanova et al., 2011). Currently, five PIDs with impaired TLR signaling pathways have been described: autosomal-recessive MyD88, IRAK4, and UNC93B1 deficiency and autosomal-dominant TLR3 and TRAF3 deficiency (Casrouge et al., 2006; Pérez de Diego et al., 2010; Picard et al., 2003; von Bernuth et al., 2008; Zhang et al., 2007). MyD88 and IRAK4 deficiencies predispose patients to recurrent life-threatening pyogenic bacterial infections, including invasive pneumococcal diseases, in childhood associated with poor inflammatory responses. The clinical symptoms of these patients are improved with age, suggesting that adaptive immunity or other PRRs may compensate the deficiency of TLR signaling. TLR3, UNC93B1, or TRAF3 deficiency specifically predisposes patients to herpes simplex virus-1 encephalitis (HSE). Although TRAF3 is involved in signaling through TNF receptor family members, a clinical phenotype of TRAF3 deficiency is probably a result from the impaired TLR3-dependent type I IFN induction, suggesting that TLR3-TRAF3 axis is indispensable in prevention against HSV-1 infection in the CNS. Whereas UNC93B1-deficient patients are prone to HSE and fail to induce type I IFN in response to TLR3, TLR7, and TLR9 ligands, IRAK4- and MyD88-deficient patients who are unresponsive to TLR7 and TLR9 ligands are otherwise normal against virus infection, including HSV-1, suggesting that the TLR3-dependent pathway plays a key role in UNC93B1-associated diseases in humans.

It has been reported that there are associations between TLR genetic variations and susceptibility or resistance to infections (Casanova et al., 2011). TLR4 polymorphism Asp299Gly allele increases the risk of Gram-negative bacteria infection and sepsis. In addition, TLR4 polymorphism is associated with an increased risk of asperigillosis, RSV bronchiolitis, and severe malaria. On the contrary, the same polymorphism increased resistance to Legionnaires diseases caused by *Legionella pneumophila*. TLR5 polymorphism Arg393STOP is oppositely found to be associated with susceptibility to Legionnaires diseases. TLR2 polymorphism Arg753Gln, which impairs downstream signaling, is associated with susceptibility to tuberculosis and with the protection from late-stage Lyme disease caused by the bacterium *Borrelia burgdorferi*. TLR9 Thr1486Cys polymorphism is found to increase the risk of severe malaria. Moreover, heterozygous of TIRAP Ser180Lue variant is reported to mediate protection against invasive pneumococcal disease, bacteremia, malaria, and tuberculosis.

Future Perspectives

Over the past decade, much progress has been made in our understanding of how the innate immune system senses and responds to microbial pathogens. The mechanisms underlying the ligand specificity, signaling pathways, and cellular trafficking of TLRs have been extensively characterized. However, microbial pathogens consist of multiple PAMPs, which activate both TLRs and other PRRs, and it is now clear that crosstalk between them is a prerequisite for the induction of effective innate immune responses. However, because of the complexity of these systems, we still know relatively little about innate immune recognition of microbial pathogens and the crosstalk between the different PRRs. Moreover, we also know little about mechanisms of how the innate immune system controls the induction of adaptive immunity. Thus, comprehensive analysis by means of mice deficient in each PRR, or in a combination of different PRRs, will be required before we fully understand the complex processes involved.

REFERENCES

Adachi, K., Tsutsui, H., Kashiwamura, S., Seki, E., Nakano, H., Takeuchi, O., Takeda, K., Okumura, K., Van Kaer, L., Okamura, H., et al. (2001). *Plasmodium berghei* infection in mice induces liver injury by an IL-12- and toll-like receptor/myeloid differentiation factor 88-dependent mechanism. J. Immunol. *167*, 5928–5934.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell *124*, 783–801.

Andersen-Nissen, E., Hawn, T.R., Smith, K.D., Nachman, A., Lampano, A.E., Uematsu, S., Akira, S., and Aderem, A. (2007). Cutting edge: Tlr5-/- mice are more susceptible to *Escherichia coli* urinary tract infection. J. Immunol. *178*, 4717–4720.

Arpaia, N., Godec, J., Lau, L., Sivick, K.E., McLaughlin, L.M., Jones, M.B., Dracheva, T., Peterson, S.N., Monack, D.M., and Barton, G.M. (2011). TLR signaling is required for *Salmonella typhimurium* virulence. Cell *144*, 675–688.

Bafica, A., Santiago, H.C., Goldszmid, R., Ropert, C., Gazzinelli, R.T., and Sher, A. (2006). Cutting edge: TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia in *Trypanosoma cruzi* infection. J. Immunol. *177*, 3515–3519.

Barbalat, R., Lau, L., Locksley, R.M., and Barton, G.M. (2009). Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. Nat. Immunol. *10*, 1200–1207.

Barbalat, R., Ewald, S.E., Mouchess, M.L., and Barton, G.M. (2011). Nucleic acid recognition by the innate immune system. Annu. Rev. Immunol. *29*, 185–214.

Barber, G.N. (2011). Innate immune DNA sensing pathways: STING, AIMII and the regulation of interferon production and inflammatory responses. Curr. Opin. Immunol. *23*, 10–20.

Barton, G.M., and Kagan, J.C. (2009). A cell biological view of Toll-like receptor function: regulation through compartmentalization. Nat. Rev. Immunol. *9*, 535–542.

Bellocchio, S., Montagnoli, C., Bozza, S., Gaziano, R., Rossi, G., Mambula, S.S., Vecchi, A., Mantovani, A., Levitz, S.M., and Romani, L. (2004). The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens in vivo. J. Immunol. *172*, 3059–3069.

Beutler, B.A. (2009). TLRs and innate immunity. Blood *113*, 1399–1407.

Biondo, C., Signorino, G., Costa, A., Midiri, A., Gerace, E., Galbo, R., Bellantoni, A., Malara, A., Beninati, C., Teti, G., and Mancuso, G. (2011). Recognition of yeast nucleic acids triggers a host protective type I interferon response. Eur. J. Immunol., in press. Published online April 11, 2011.

Blasius, A.L., and Beutler, B. (2010). Intracellular toll-like receptors. Immunity *32*, 305–315.

Bourgeois, C., Majer, O., Frohner, I.E., Lesiak-Markowicz, I., Hildering, K.S., Glaser, W., Stockinger, S., Decker, T., Akira, S., Müller, M., and Kuchler, K. (2011). Conventional dendritic cells mount a type I IFN response against *Candida* spp. requiring novel phagosomal TLR7-mediated IFN-beta signaling. J. Immunol. *186*, 3104–3112.

Bozza, S., Gaziano, R., Lipford, G.B., Montagnoli, C., Bacci, A., Di Francesco, P., Kurup, V.P., Wagner, H., and Romani, L. (2002). Vaccination of mice against invasive aspergillosis with recombinant *Aspergillus* proteins and CpG oligodeoxynucleotides as adjuvants. Microbes Infect. *4*, 1281–1290.

Brinkmann, M.M., Spooner, E., Hoebe, K., Beutler, B., Ploegh, H.L., and Kim, Y.M. (2007). The interaction between the ER membrane protein UNC93B and TLR3, 7, and 9 is crucial for TLR signaling. J. Cell Biol. *177*, 265–275.

Casanova, J.L., Abel, L., and Quintana-Murci, L. (2011). Human TLRs and IL-1Rs in host defense: Natural insights from evolutionary, epidemiological, and clinical genetics. Annu. Rev. Immunol. *29*, 447–491.

Casrouge, A., Zhang, S.Y., Eidenschenk, C., Jouanguy, E., Puel, A., Yang, K., Alcais, A., Picard, C., Mahfoufi, N., Nicolas, N., et al. (2006). Herpes simplex virus encephalitis in human UNC-93B deficiency. Science *314*, 308–312.

Choi, Y.J., Im, E., Chung, H.K., Pothoulakis, C., and Rhee, S.H. (2010). TRIF mediates Toll-like receptor 5-induced signaling in intestinal epithelial cells. J. Biol. Chem. *285*, 37570–37578.

Coban, C., Ishii, K.J., Kawai, T., Hemmi, H., Sato, S., Uematsu, S., Yamamoto, M., Takeuchi, O., Itagaki, S., Kumar, N., et al. (2005). Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. J. Exp. Med. *201*, 19–25.

Coban, C., Ishii, K.J., Uematsu, S., Arisue, N., Sato, S., Yamamoto, M., Kawai, T., Takeuchi, O., Hisaeda, H., Horii, T., and Akira, S. (2007). Pathological role of Toll-like receptor signaling in cerebral malaria. Int. Immunol. *19*, 67–79.

Coban, C., Igari, Y., Yagi, M., Reimer, T., Koyama, S., Aoshi, T., Ohata, K., Tsukui, T., Takeshita, F., Sakurai, K., et al. (2010). Immunogenicity of whole-parasite vaccines against *Plasmodium falciparum* involves malarial hemozoin and host TLR9. Cell Host Microbe *7*, 50–61.

Daffis, S., Samuel, M.A., Suthar, M.S., Gale, M., Jr., and Diamond, M.S. (2008). Toll-like receptor 3 has a protective role against West Nile virus infection. J. Virol. *82*, 10349–10358.

Dorhoi, A., Desel, C., Yeremeev, V., Pradl, L., Brinkmann, V., Mollenkopf, H.J., Hanke, K., Gross, O., Ruland, J., and Kaufmann, S.H. (2010). The adaptor molecule CARD9 is essential for tuberculosis control. J. Exp. Med. *207*, 777–792.

Dostert, C., Guarda, G., Romero, J.F., Menu, P., Gross, O., Tardivel, A., Suva, M.L., Stehle, J.C., Kopf, M., Stamenkovic, I., et al. (2009). Malarial hemozoin is a Nalp3 inflammasome activating danger signal. PLoS ONE *4*, e6510.

Edelmann, K.H., Richardson-Burns, S., Alexopoulou, L., Tyler, K.L., Flavell, R.A., and Oldstone, M.B. (2004). Does Toll-like receptor 3 play a biological role in virus infections? Virology *322*, 231–238.

Edwards, A.D., Diebold, S.S., Slack, E.M., Tomizawa, H., Hemmi, H., Kaisho, T., Akira, S., and Reis e Sousa, C. (2003). Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines. Eur. J. Immunol. *33*, 827–833.

Elinav, E., Strowig, T., Henao-Mejia, J., and Flavell, R.A. (2011). Regulation of the antimicrobial response by NLR proteins. Immunity *34*, this issue, 665–679.

Ewald, S.E., Engel, A., Lee, J., Wang, M., Bogyo, M., and Barton, G.M. (2011). Nucleic acid recognition by Toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. J. Exp. Med. *208*, 643–651.

Fernandes-Alnemri, T., Yu, J.W., Juliana, C., Solorzano, L., Kang, S., Wu, J., Datta, P., McCormick, M., Huang, L., McDermott, E., et al. (2010). The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. Nat. Immunol. *11*, 385–393.

Feuillet, V., Medjane, S., Mondor, I., Demaria, O., Pagni, P.P., Galán, J.E., Flavell, R.A., and Alexopoulou, L. (2006). Involvement of Toll-like receptor 5 in the recognition of flagellated bacteria. Proc. Natl. Acad. Sci. USA *103*, 12487–12492.

Fremond, C.M., Togbe, D., Doz, E., Rose, S., Vasseur, V., Maillet, I., Jacobs, M., Ryffel, B., and Quesniaux, V.F. (2007). IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to *Mycobacterium tuberculosis* infection. J. Immunol. *179*, 1178–1189.

Fukui, R., Saitoh, S., Matsumoto, F., Kozuka-Hata, H., Oyama, M., Tabeta, K., Beutler, B., and Miyake, K. (2009). Unc93B1 biases Toll-like receptor responses to nucleic acid in dendritic cells toward DNA- but against RNA-sensing. J. Exp. Med. *206*, 1339–1350.

Gandotra, S., Jang, S., Murray, P.J., Salgame, P., and Ehrt, S. (2007). Nucleotide-binding oligomerization domain protein 2-deficient mice control infection with *Mycobacterium tuberculosis*. Infect. Immun. *75*, 5127–5134.

Gavin, A.L., Hoebe, K., Duong, B., Ota, T., Martin, C., Beutler, B., and Nemazee, D. (2006). Adjuvant-enhanced antibody responses in the absence of toll-like receptor signaling. Science *314*, 1936–1938.

Gazzinelli, R.T., and Denkers, E.Y. (2006). Protozoan encounters with Toll-like receptor signalling pathways: Implications for host parasitism. Nat. Rev. Immunol. *6*, 895–906.

Geijtenbeek, T.B., and Gringhuis, S.I. (2009). Signalling through C-type lectin receptors: Shaping immune responses. Nat. Rev. Immunol. *9*, 465–479.

Gerold, G., Zychlinsky, A., and de Diego, J.L. (2007). What is the role of Toll-like receptors in bacterial infections? Semin. Immunol. *19*, 41–47.

Gilliet, M., Cao, W., and Liu, Y.J. (2008). Plasmacytoid dendritic cells: Sensing nucleic acids in viral infection and autoimmune diseases. Nat. Rev. Immunol. *8*, 594–606.

Glocker, E.O., Hennigs, A., Nabavi, M., Schäffer, A.A., Woellner, C., Salzer, U., Pfeifer, D., Veelken, H., Warnatz, K., Tahami, F., et al. (2009). A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N. Engl. J. Med. *361*, 1727–1735.

Goodridge, H.S., and Underhill, D.M. (2008). Fungal recognition by TLR2 and Dectin-1. Handb Exp Pharmacol *183*, 87–109.

Gotoh, K., Tanaka, Y., Nishikimi, A., Nakamura, R., Yamada, H., Maeda, N., Ishikawa, T., Hoshino, K., Uruno, T., Cao, Q., et al. (2010). Selective control of type I IFN induction by the Rac activator DOCK2 during TLR-mediated plasmacytoid dendritic cell activation. J. Exp. Med. *207*, 721–730.

Griffith, J.W., Sun, T., McIntosh, M.T., and Bucala, R. (2009). Pure Hemozoin is inflammatory in vivo and activates the NALP3 inflammasome via release of uric acid. J. Immunol. *183*, 5208–5220.

Gross, O., Gewies, A., Finger, K., Schäfer, M., Sparwasser, T., Peschel, C., Förster, I., and Ruland, J. (2006). Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature *442*, 651–656.

Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hannesschläger, N., Endres, S., Hartmann, G., Tardivel, A., Schweighoffer, E., Tybulewicz, V., et al. (2009). Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. Nature *459*, 433–436.

Haas, T., Metzger, J., Schmitz, F., Heit, A., Müller, T., Latz, E., and Wagner, H. (2008). The DNA sugar backbone 2′ deoxyribose determines toll-like receptor 9 activation. Immunity *28*, 315–323.

Hisaeda, H., Tetsutani, K., Imai, T., Moriya, C., Tu, L., Hamano, S., Duan, X., Chou, B., Ishida, H., Aramaki, A., et al. (2008). Malaria parasites require TLR9 signaling for immune evasion by activating regulatory T cells. J. Immunol. *180*, 2496–2503.

Hise, A.G., Tomalka, J., Ganesan, S., Patel, K., Hall, B.A., Brown, G.D., and Fitzgerald, K.A. (2009). An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. Cell Host Microbe *5*, 487–497.

Hoffmann, J.A. (2003). The immune response of *Drosophila*. Nature *426*, 33–38.

Hölscher, C., Reiling, N., Schaible, U.E., Hölscher, A., Bathmann, C., Korbel, D., Lenz, I., Sonntag, T., Kröger, S., Akira, S., et al. (2008). Containment of aerogenic *Mycobacterium tuberculosis* infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. Eur. J. Immunol. *38*, 680–694.

Honda, K., Ohba, Y., Yanai, H., Negishi, H., Mizutani, T., Takaoka, A., Taya, C., and Taniguchi, T. (2005). Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. Nature *434*, 1035–1040.

Horner, S.M., and Gale, M., Jr. (2009). Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. J. Interferon Cytokine Res. *29*, 489–498.

Hou, B., Benson, A., Kuzmich, L., DeFranco, A.L., and Yarovinsky, F. (2011). Critical coordination of innate immune defense against *Toxoplasma gondii* by dendritic cells responding via their Toll-like receptors. Proc. Natl. Acad. Sci. USA *108*, 278–283.

Husebye, H., Aune, M.H., Stenvik, J., Samstad, E., Skjeldal, F., Halaas, O., Nilsen, N.J., Stenmark, H., Latz, E., Lien, E., et al. (2010). The Rab11a GTPase controls Toll-like receptor 4-induced activation of interferon regulatory factor-3 on phagosomes. Immunity *33*, 583–596.

Ichinohe, T. (2010). Respective roles of TLR, RIG-I and NLRP3 in influenza virus infection and immunity: Impact on vaccine design. Expert Rev. Vaccines *9*, 1315–1324.

Imai, Y., Kuba, K., Neely, G.G., Yaghubian-Malhami, R., Perkmann, T., van Loo, G., Ermolaeva, M., Veldhuizen, R., Leung, Y.H., Wang, H., et al. (2008). Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell *133*, 235–249.

Ishii, K.J., Kawagoe, T., Koyama, S., Matsui, K., Kumar, H., Kawai, T., Uematsu, S., Takeuchi, O., Takeshita, F., Coban, C., and Akira, S. (2008). TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature *451*, 725–729.

Ishikawa, E., Ishikawa, T., Morita, Y.S., Toyonaga, K., Yamada, H., Takeuchi, O., Kinoshita, T., Akira, S., Yoshikai, Y., and Yamasaki, S. (2009). Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J. Exp. Med. *206*, 2879–2888.

Janeway, C.A., Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. *54*, 1–13.

Joly, S., Ma, N., Sadler, J.J., Soll, D.R., Cassel, S.L., and Sutterwala, F.S. (2009). Cutting edge: *Candida albicans* hyphae formation triggers activation of the Nlrp3 inflammasome. J. Immunol. *183*, 3578–3581.

Kanto, T., Inoue, M., Miyatake, H., Sato, A., Sakakibara, M., Yakushijin, T., Oki, C., Itose, I., Hiramatsu, N., Takehara, T., et al. (2004). Reduced numbers and impaired ability of myeloid and plasmacytoid dendritic cells to polarize T helper cells in chronic hepatitis C virus infection. J. Infect. Dis. *190*, 1919–1926.

Kawai, T., and Akira, S. (2006). Innate immune recognition of viral infection. Nat. Immunol. *7*, 131–137.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. Nat. Immunol. *11*, 373–384.

Kim, Y.M., Brinkmann, M.M., Paquet, M.E., and Ploegh, H.L. (2008). UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes. Nature *452*, 234–238.

Koyama, S., Ishii, K.J., Kumar, H., Tanimoto, T., Coban, C., Uematsu, S., Kawai, T., and Akira, S. (2007). Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination. J. Immunol. *179*, 4711–4720.

Koyama, S., Aoshi, T., Tanimoto, T., Kumagai, Y., Kobiyama, K., Tougan, T., Sakurai, K., Coban, C., Horii, T., Akira, S., and Ishii, K.J. (2010). Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes. Sci. Transl. Med. *2*, ra24.

Kropf, P., Freudenberg, M.A., Modolell, M., Price, H.P., Herath, S., Antoniazi, S., Galanos, C., Smith, D.F., and Müller, I. (2004). Toll-like receptor 4 contributes to efficient control of infection with the protozoan parasite *Leishmania major*. Infect. Immun. *72*, 1920–1928.

Kumagai, Y., Takeuchi, O., Kato, H., Kumar, H., Matsui, K., Morii, E., Aozasa, K., Kawai, T., and Akira, S. (2007). Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses. Immunity *27*, 240–252.

Kumar, H., Koyama, S., Ishii, K.J., Kawai, T., and Akira, S. (2008). Cutting edge: Cooperation of IPS-1- and TRIF-dependent pathways in poly IC-enhanced antibody production and cytotoxic T cell responses. J. Immunol. *180*, 683–687.

Kumar, H., Kumagai, Y., Tsuchida, T., Koenig, P.A., Satoh, T., Guo, Z., Jang, M.H., Saitoh, T., Akira, S., and Kawai, T. (2009). Involvement of the NLRP3 inflammasome in innate and humoral adaptive immune responses to fungal beta-glucan. J. Immunol. *183*, 8061–8067.

Lamkanfi, M., Malireddi, R.K., and Kanneganti, T.D. (2009). Fungal zymosan and mannan activate the cryopyrin inflammasome. J. Biol. Chem. *284*, 20574–20581.

Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., Escriou, N., Flavell, R., Chignard, M., and Si-Tahar, M. (2006). Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog. *2*, e53.

Lee, H.K., Lund, J.M., Ramanathan, B., Mizushima, N., and Iwasaki, A. (2007). Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science *315*, 1398–1401.

Lemon, S.M. (2010). Induction and evasion of innate antiviral responses by hepatitis C virus. J. Biol. Chem. *285*, 22741–22747.

Letran, S.E., Lee, S.J., Atif, S.M., Uematsu, S., Akira, S., and McSorley, S.J. (2011). TLR5 functions as an endocytic receptor to enhance flagellin-specific adaptive immunity. Eur. J. Immunol. *41*, 29–38.

Longhi, M.P., Trumpfheller, C., Idoyaga, J., Caskey, M., Matos, I., Kluger, C., Salazar, A.M., Colonna, M., and Steinman, R.M. (2009). Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. J. Exp. Med. *206*, 1589–1602.

Loo, Y.-M., and Gale, M., Jr. (2011). Immune signaling by RIG-I-like receptors. Immunity *34*, this issue, 680–692.

Mancuso, G., Gambuzza, M., Midiri, A., Biondo, C., Papasergi, S., Akira, S., Teti, G., and Beninati, C. (2009). Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells. Nat. Immunol. *10*, 587–594.

Marakalala, M.J., Graham, L.M., and Brown, G.D. (2010). The role of Syk/CARD9-coupled C-type lectin receptors in immunity to *Mycobacterium tuberculosis* infections. Clin. Dev. Immunol. *2010*, 567571.

Mayer-Barber, K.D., Barber, D.L., Shenderov, K., White, S.D., Wilson, M.S., Cheever, A., Kugler, D., Hieny, S., Caspar, P., Núñez, G., et al. (2010). Caspase-1 independent IL-1beta production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling in vivo. J. Immunol. *184*, 3326–3330.

McElvania Tekippe, E., Allen, I.C., Hulseberg, P.D., Sullivan, J.T., McCann, J.R., Sandor, M., Braunstein, M., and Ting, J.P. (2010). Granuloma formation and host defense in chronic *Mycobacterium tuberculosis* infection requires PYCARD/ASC but not NLRP3 or caspase-1. PLoS ONE *5*, e12320.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. Nature *449*, 819–826.

Melo, M.B., Kasperkovitz, P., Cerny, A., Könen-Waisman, S., Kurt-Jones, E.A., Lien, E., Beutler, B., Howard, J.C., Golenbock, D.T., and Gazzinelli, R.T. (2010). UNC93B1 mediates host resistance to infection with *Toxoplasma gondii*. PLoS Pathog. *6*, e1001071.

Miao, E.A., Andersen-Nissen, E., Warren, S.E., and Aderem, A. (2007). TLR5 and Ipaf: Dual sensors of bacterial flagellin in the innate immune system. Semin. Immunopathol. *29*, 275–288.

Miller, L.S., O'Connell, R.M., Gutierrez, M.A., Pietras, E.M., Shahangian, A., Gross, C.E., Thirumala, A., Cheung, A.L., Cheng, G., and Modlin, R.L. (2006). MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against *Staphylococcus aureus*. Immunity *24*, 79–91.

Mócsai, A., Ruland, J., and Tybulewicz, V.L. (2010). The SYK tyrosine kinase: A crucial player in diverse biological functions. Nat. Rev. Immunol. *10*, 387–402.

Mun, H.S., Aosai, F., Norose, K., Chen, M., Piao, L.X., Takeuchi, O., Akira, S., Ishikura, H., and Yano, A. (2003). TLR2 as an essential molecule for protective immunity against *Toxoplasma gondii* infection. Int. Immunol. *15*, 1081–1087.

Negishi, H., Osawa, T., Ogami, K., Ouyang, X., Sakaguchi, S., Koshiba, R., Yanai, H., Seko, Y., Shitara, H., Bishop, K., et al. (2008). A critical link between Toll-like receptor 3 and type II interferon signaling pathways in antiviral innate immunity. Proc. Natl. Acad. Sci. USA *105*, 20446–20451.

Netea, M.G., and Maródi, L. (2010). Innate immune mechanisms for recognition and uptake of *Candida* species. Trends Immunol. *31*, 346–353.

Netea, M.G., Sutmuller, R., Hermann, C., Van der Graaf, C.A., Van der Meer, J.W., van Krieken, J.H., Hartung, T., Adema, G., and Kullberg, B.J. (2004). Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. J. Immunol. *172*, 3712–3718.

Osorio, F., and Reis e Sousa, C. (2011). Myeloid C-type lectin receptors in pathogen recogniztion and host defense. Immunity *34*, this issue, 651–664.

Palsson-McDermott, E.M., Doyle, S.L., McGettrick, A.F., Hardy, M., Husebye, H., Banahan, K., Gong, M., Golenbock, D., Espevik, T., and O'Neill, L.A. (2009). TAG, a splice variant of the adaptor TRAM, negatively regulates the adaptor MyD88-independent TLR4 pathway. Nat. Immunol. *10*, 579–586.

Park, B., Buti, L., Lee, S., Matsuwaki, T., Spooner, E., Brinkmann, M.M., Nishihara, M., and Ploegh, H.L. (2011). Granulin is a soluble cofactor for Toll-like Receptor 9 signaling. Immunity *34*, 505–513.

Pérez de Diego, R., Sancho-Shimizu, V., Lorenzo, L., Puel, A., Plancoulaine, S., Picard, C., Herman, M., Cardon, A., Durandy, A., Bustamante, J., et al. (2010).

Human TRAF3 adaptor molecule deficiency leads to impaired Toll-like receptor 3 response and susceptibility to herpes simplex encephalitis. Immunity *33*, 400–411.

Picard, C., Puel, A., Bonnet, M., Ku, C.L., Bustamante, J., Yang, K., Soudais, C., Dupuis, S., Feinberg, J., Fieschi, C., et al. (2003). Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science *299*, 2076–2079.

Pifer, R., Benson, A., Sturge, C.R., and Yarovinsky, F. (2011). UNC93B1 is essential for TLR11 activation and IL-12-dependent host resistance to *Toxoplasma gondii*. J. Biol. Chem. *286*, 3307–3314.

Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hannesschläger, N., Schlee, M., Rothenfusser, S., Barchet, W., et al. (2010). Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. Nat. Immunol. *11*, 63–69.

Rathinam, V.A., Jiang, Z., Waggoner, S.N., Sharma, S., Cole, L.E., Waggoner, L., Vanaja, S.K., Monks, B.G., Ganesan, S., Latz, E., et al. (2010). The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat. Immunol. *11*, 395–402.

Rehermann, B. (2009). Hepatitis C virus versus innate and adaptive immune responses: A tale of coevolution and coexistence. J. Clin. Invest. *119*, 1745–1754.

Reizis, B., Bunin, A., Ghosh, H.S., Lewis, K.L., and Sisirak, V. (2011). Plasmacytoid dendritic cells: recent progress and open questions. Annu. Rev. Immunol. *29*, 163–183.

Romani, L. (2011). Immunity to fungal infections. Nat. Rev. Immunol. *11*, 275–288.

Ropert, C., and Gazzinelli, R.T. (2004). Regulatory role of Toll-like receptor 2 during infection with *Trypanosoma cruzi*. J. Endotoxin Res. *10*, 425–430.

Saiga, H., Shimada, Y., and Takeda, K. (2011). Innate immune effectors in mycobacterial infection. Clin. Dev. Immunol. *2011*, 347594.

Saitoh, S., and Miyake, K. (2009). Regulatory molecules required for nucleotide-sensing Toll-like receptors. Immunol. Rev. *227*, 32–43.

Saitoh, T., Satoh, T., Yamamoto, N., Uematsu, S., Takeuchi, O., Kawai, T., and Akira, S. (2011). Antiviral protein Viperin promotes Toll-like receptor 7- and Toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. Immunity *34*, 352–363.

Sasai, M., Linehan, M.M., and Iwasaki, A. (2010). Bifurcation of Toll-like receptor 9 signaling by adaptor protein 3. Science *329*, 1530–1534.

Schoenen, H., Bodendorfer, B., Hitchens, K., Manzanero, S., Werninghaus, K., Nimmerjahn, F., Agger, E.M., Stenger, S., Andersen, P., Ruland, J., et al. (2010). Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J. Immunol. *184*, 2756–2760.

Schulz, O., Diebold, S.S., Chen, M., Näslund, T.I., Nolte, M.A., Alexopoulou, L., Azuma, Y.T., Flavell, R.A., Liljeström, P., and Reis e Sousa, C. (2005). Toll-like receptor 3 promotes cross-priming to virus-infected cells. Nature *433*, 887–892.

Seo, S.U., Kwon, H.J., Song, J.H., Byun, Y.H., Seong, B.L., Kawai, T., Akira, S., and Kweon, M.N. (2010). MyD88 signaling is indispensable for primary influenza A virus infection but dispensable for secondary infection. J. Virol. *84*, 12713–12722.

Shi, Z., Cai, Z., Sanchez, A., Zhang, T., Wen, S., Wang, J., Yang, J., Fu, S., and Zhang, D. (2011). A novel Toll-like receptor that recognizes vesicular stomatitis virus. J. Biol. Chem. *286*, 4517–4524.

Shiina, M., and Rehermann, B. (2008). Cell culture-produced hepatitis C virus impairs plasmacytoid dendritic cell function. Hepatology *47*, 385–395.

Shio, M.T., Eisenbarth, S.C., Savaria, M., Vinet, A.F., Bellemare, M.J., Harder, K.W., Sutterwala, F.S., Bohle, D.S., Descoteaux, A., Flavell, R.A., and Olivier, M. (2009). Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. PLoS Pathog. *5*, e1000559.

Spreafico, R., Ricciardi-Castagnoli, P., and Mortellaro, A. (2010). The controversial relationship between NLRP3, alum, danger signals and the next-generation adjuvants. Eur. J. Immunol. *40*, 638–642.

Sutmuller, R.P., den Brok, M.H., Kramer, M., Bennink, E.J., Toonen, L.W., Kullberg, B.J., Joosten, L.A., Akira, S., Netea, M.G., and Adema, G.J. (2006). Toll-like receptor 2 controls expansion and function of regulatory T cells. J. Clin. Invest. *116*, 485–494.

Tabeta, K., Georgel, P., Janssen, E., Du, X., Hoebe, K., Crozat, K., Mudd, S., Shamel, L., Sovath, S., Goode, J., et al. (2004). Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc. Natl. Acad. Sci. USA *101*, 3516–3521.

Tabeta, K., Hoebe, K., Janssen, E.M., Du, X., Georgel, P., Crozat, K., Mudd, S., Mann, N., Sovath, S., Goode, J., et al. (2006). The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. Nat. Immunol. *7*, 156–164.

Takahashi, K., Asabe, S., Wieland, S., Garaigorta, U., Gastaminza, P., Isogawa, M., and Chisari, F.V. (2010). Plasmacytoid dendritic cells sense hepatitis C virus-infected cells, produce interferon, and inhibit infection. Proc. Natl. Acad. Sci. USA *107*, 7431–7436.

Takeuchi, O., Hoshino, K., and Akira, S. (2000). Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. J. Immunol. *165*, 5392–5396.

Uematsu, S., Jang, M.H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., Kato, H., Sougawa, N., Matsui, H., Kuwata, H., et al. (2006). Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. Nat. Immunol. *7*, 868–874.

Uematsu, S., Fujimoto, K., Jang, M.H., Yang, B.G., Jung, Y.J., Nishiyama, M., Sato, S., Tsujimura, T., Yamamoto, M., Yokota, Y., et al. (2008). Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat. Immunol. *9*, 769–776.

Verdrengh, M., Thomas, J.A., and Hultgren, O.H. (2004). IL-1 receptor-associated kinase 1 mediates protection against *Staphylococcus aureus* infection. Microbes Infect. *6*, 1268–1272.

Vijay-Kumar, M., Carvalho, F.A., Aitken, J.D., Fifadara, N.H., and Gewirtz, A.T. (2010). TLR5 or NLRC4 is necessary and sufficient for promotion of humoral immunity by flagellin. Eur. J. Immunol. *40*, 3528–3534.

Villadangos, J.A., and Shortman, K. (2010). Found in translation: The human equivalent of mouse CD8+ dendritic cells. J. Exp. Med. *207*, 1131–1134.

von Bernuth, H., Picard, C., Jin, Z., Pankla, R., Xiao, H., Ku, C.L., Chrabieh, M., Mustapha, I.B., Ghandil, P., Camcioglu, Y., et al. (2008). Pyogenic bacterial infections in humans with MyD88 deficiency. Science *321*, 691–696.

Wang, T., Town, T., Alexopoulou, L., Anderson, J.F., Fikrig, E., and Flavell, R.A. (2004). Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat. Med. *10*, 1366–1373.

Weiss, D.S., Raupach, B., Takeda, K., Akira, S., and Zychlinsky, A. (2004). Toll-like receptors are temporally involved in host defense. J. Immunol. *172*, 4463–4469.

Werninghaus, K., Babiak, A., Gross, O., Hölscher, C., Dietrich, H., Agger, E.M., Mages, J., Mocsai, A., Schoenen, H., Finger, K., et al. (2009). Adjuvanticity of a synthetic cord factor analogue for subunit *Mycobacterium tuberculosis* vaccination requires FcRgamma-Syk-Card9-dependent innate immune activation. J. Exp. Med. *206*, 89–97.

West, A.P., Brodsky, I.E., Rahner, C., Woo, D.K., Erdjument-Bromage, H., Tempst, P., Walsh, M.C., Choi, Y., Shadel, G.S., and Ghosh, S. (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature *472*, 476–480.

Yanai, H., Ban, T., Wang, Z., Choi, M.K., Kawamura, T., Negishi, H., Nakasato, M., Lu, Y., Hangai, S., Koshiba, R., et al. (2009). HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature *462*, 99–103.

Yarovinsky, F., Zhang, D., Andersen, J.F., Bannenberg, G.L., Serhan, C.N., Hayden, M.S., Hieny, S., Sutterwala, F.S., Flavell, R.A., Ghosh, S., and Sher, A. (2005). TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science *308*, 1626–1629.

Zhang, S.Y., Jouanguy, E., Ugolini, S., Smahi, A., Elain, G., Romero, P., Segal, D., Sancho-Shimizu, V., Lorenzo, L., Puel, A., et al. (2007). TLR3 deficiency in patients with herpes simplex encephalitis. Science *317*, 1522–1527.