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Immunity
Review

When Signaling Pathways Collide: Positive and Negative Regulation of Toll-like Receptor Signal Transduction

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Toll-like receptor (TLR) signaling is subjected to crosstalk from other signals, with a resulting positive or negative effect. There is complex crosstalk between the NLR family of immune-regulatory molecules and TLRs, and C-type lectin receptors such as Dectin-1 synergize with TLR2 via the tyrosine kinase Syk. Bruton's tyrosine kinase plays an important positive role in TLR signaling, whereas the TAM family of receptor tyrosine kinases is inhibitory. The tyrosine phosphatase SHP1 has been shown to positively regulate induction of interferon-β, whereas SHP2 inhibits the kinase TBK1, limiting this response. K63-linked polyubiquination has also been shown to be critical for the initiation of TLR signaling. Finally, glucocorticoids affect TLR signaling by inducing the phosphatase MKP1 and inhibiting TBK1 activation. These recent findings emphasize the importance of considering TLR signaling in the context of other signaling pathways, as is likely to occur in vivo during infection and inflammation.

In order to have a molecular understanding of the immune response, we need a detailed description of signaling pathways activated by the multitude of sensors of the products of microbes. These products can be peptides presented via the major histocompatibility complex (MHC) in antigen-presenting cells to T lymphocytes. Arguably more is known about the biochemical events that occur in T cells when the T cell receptor is activated (and associated costimulatory receptors) than any other cell in the body. Signaling in T cells has been a major focus for molecular immunologists, and for good reason, given the central importance of T cells for host defense and the ongoing complexities of T cell subsets being revealed in different contexts.

There has also been an explosion of information on receptors on front-line host defense cells such as macrophages and dendritic cells (DCs), which when activated lead to a change in gene expression. The genes that are expressed code for proteins important for inflammation, for innate immunity, and also for the instruction of T and B cells in the adaptive arm of immunity. These receptors are grouped together under the heading of innate immunity because there is no rearrangement of gene segments in their generation and no apparent memory response involving the cells that express them. Much progress has been made on how these innate sensors signal. However it has also become apparent that there is complex positive and negative regulation of the signaling pathways activated as a result of crosstalk with other signals. This review will discuss the recent progress in our understanding of this aspect of innate signaling. These advances will be important for our overall understanding of the molecular regulation of innate immunity during infection and inflammation.

Types of Innate Sensors

The best-known class are the Toll-like receptors (TLRs), and other classes include the NOD-like receptors (or recently defined as nucleotide-binding domain and leucine-rich repeat containing molecules [NLRs]), RIG-I-like receptors (RLRs), and C-type lectin receptors (CLRs) (Creagh and O'Neill, 2006; Willment and Brown, 2008). All of these respond to various microbial products, with the TLRs providing a repertoire to respond to all pathogens, be they bacterial, viral, fungal, or parasitic (Bowie and O'Neill, 2007). The best characterized are TLR2, which in combination with TLR1 or TLR6 can recognize acylated lipopeptides; TLR3, which senses double-stranded RNA; TLR4, which senses LPS; and TLR9, which senses hypomethylated CpG motifs.

NLRs that have been assigned functions include NOD1 and NOD2, which sense iE-DAP and MDP, respectively (Kanneganti et al., 2007b). Both of these are breakdown products of peptidoglycan. In addition, Nalp3 (also called Cryopyrin of Nlrp3) senses multiple pathogens, pathogen products such as MDP, and products of damaged cells such as uric acid crystals and exogenous crystals such as asbestos (Martinon et al., 2004, 2006; Dostert et al., 2008). The best-known RLR is RIG-I, which senses single-stranded RNA found in viruses (Yoneyama et al., 2004), and the best known CLR is Dectin-1, which senses beta-glucan, found in fungal cell walls (Brown et al., 2002).

Signaling Components of the Innate Sensors

An important signal activated by TLRs, NOD1, NOD2, and Dectin-1 is the transcription factor NF-κB, which regulates the expression of many immune and inflammatory genes (Bowie and O'Neill, 2007). They also activate the MAP kinases p38 and ERK1 and/or ERK2, which also play a role in enhanced gene transcription and, in the case of p38, the stability of induced mRNA species containing AU repeats in their 3′ ends. Nalp3, in contrast, is found in an inflammasome with caspase-1 and is important for caspase-1 activation (Martinon, 2008). This is the enzyme that processes the proform of IL-1β into its mature

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form. Finally, the RLRs target another transcription factor, IRF3, which is also activated by TLRs involved in viral recognition. IRF3 regulates the production of type I interferons (Yoneyama et al., 2004). IRF7 has also been shown to play an important role in the induction of type I interferons by TLRs (Honda et al., 2005).

There has been remarkable progress in understanding how these innate sensors signal. ''New'' signaling proteins, protein kinases, and transcription factors have been found, representing a major advance in knowledge in immune cell signaling. In the case of TLRs, a family of five signaling adapters was found (Bowie and O'Neill, 2007). The defining domain is the Toll-IL-1 receptor (TIR) domain, which occurs on the cytosolic face of the receptors, and also in the adapters. Recruitment of the adapters to the TLR cytosolic face allows signaling to proceed.

However, no signaling pathway occurs in isolation in cells, especially in vivo. Recently, insights into other signaling pathways that affect TLR signaling have been described. Some of these will have a positive effect and synergize (e.g., the reported synergies between NOD1 or NOD2 or Dectin-1 with certain TLRs for activation of signals such as NF-κB [Tada et al., 2005; van Heel et al., 2005; Dennehy et al., 2008]), whereas others will have a negative effect (e.g., activation of the Tyro Axl Mer (TAM) family of tyrosine kinases, which inhibits TLR signaling [Rothlin et al., 2007]). Some processes can be both positive and negative, as can be seen with ubiquination of proteins on the pathway, which can either activate (e.g., K63-linked ubiquination of TRAF6 on the NF-κB pathway (Deng et al., 2000; Wang et al., 2001) or lead to degradation (e.g., K48-linked ubiquitination of IκB). Although trying to understand a signaling pathway in isolation has its challenges, integrating multiple pathways, as would occur in vivo, provides us with a very demanding goal, which must be achieved if we are to understand signaling in health and disease.

TLR Signaling Adapters—an Update

The area of signaling by the TLR adapters has been reviewed extensively elsewhere (Bowie and O'Neill, 2007). In brief, the model currently used to describe initiation of signaling involves ligand-induced dimerization of TLRs, creating a TIR-TIR interface, which acts to recruit adapters via their TIR domains (Weber et al., 2005). In the case of TLR9, it has been shown that a pre-exising dimer occurs, which presumably ''tightens'' upon ligand binding to create a new conformation (Latz et al. (2007)). Specificity is evident in adaptor usage by different TLRs (Bowie and O'Neill, 2007). MyD88 is the universal adaptor, used by all TLRs except TLR3, and acts to recruit the IRAK family of kinases. These ultimately trigger NF-κB, p38, and ERK activation. MyD88 adaptor-like (Mal, also called TIRAP) is recruited by TLR2 and TLR4, and so far its main function appears to be to subsequently stabilize MyD88 in the complex, acting as a bridge. Trif is used by TLR3 and also TLR4, but in the case of TLR4 another bridging adaptor is needed, Tram. Trif leads to IRF3 activation via recruitment of the kinase TBK-1, and this signal, in the case of both TLR3 and TLR4, comes from the endosome, with TLR4 trafficking there after LPS recognition (Tanimura et al., 2008; Kagan et al., 2008). Finally, the adaptor SARM has been shown to inhibit Trif and therefore may be a negative regulator (Carty et al., 2006). We therefore have an increasingly clear picture of the initiation of TLR signaling.

We also have a good picture of the covalent regulation of components in TLR signaling. The role of phosphorylation by tyrosine kinases in the regulation of TLR signaling will be discussed in detail below, given recent advances. Similarly, there have been advances in the regulation of signaling by ubiquitination that will be discussed below. In addition, the cell biology of the adapters in TLR signaling is becoming clearer. For example, Mal has a phosphatidylinositol-4,5-bisphosphase (PIP2) binding domain that localizes it to the plasma membrane (Kagan and Medzhitov, 2006), whereas TRAM is myristoylated for the same reason (Rowe et al., 2006). A recent important insight into TLR4 signaling has come from the observation that it is the localization of TLR4 that determines whether the Mal-MyD88 pathway or the TRAM-TRIF pathway is activated (Tanimura et al., 2008; Kagan et al., 2008). TLR4 in the plasma membrane engages with Mal and subsequently MyD88, for NF-κB activation via TRAF6. TLR4 will then traffic to endosomes, where TRAM is now engaged, recruiting TRIF, which in turn recruits TRAF3 to activate TBK-1. Binding of Mal and TRAM appear to be mutually exclusive (Núñez Miguel et al., 2007). This gives us an explanation for how TLR4 can engage with TBK-1 because the other receptors that activate this kinase are cytosolic or endosomal (e.g., TLR3). Phosphorylation of TRAM on serine 16 by protein kinase C-epsilon is required for this process (McGettrick et al., 2006) and may be required for endosomal trafficking or displacement of Mal-MyD88 from the complex.

The broad family of TIR adapters also continues to grow. The latest mammalian member, SARM, has been shown to inhibit signaling by TRIF and is therefore a negative regulator of the IRF3 pathway (Carty et al., 2006). It has also been shown to be expressed in neurons, where it interacts with the MAP kinase JNK3 (Kim et al., 2007). It appears to have a damaging role in brain because it has been demonstrated to be involved in the death of hippocampal neurons as a result of deprivation of oxygen and glucose. How this fits with its role as a regulator of TRIF is not clear, and there may be an important species difference between mouse and human in this regard; its role in TRIF regulation may be more relevant to humans. TIR adapters also continue to be uncovered in other species, a recent and striking example being in various bacteria. The first report of this was in *Salmonella enteritica* serovar, which was shown to have a TIR-like protein that impairs TLR signaling, promoting intracellular bacterial accumulation (Newman et al., 2006). A uropathogenic strain of *E. coli*, termed CFT073, was then shown to have a TIR-domain-containing protein termed TIR-containing protein C (TcpC) (Cirl et al., 2008). The bacteria release this protein, which is somehow taken up by macrophages, where it interacts with MyD88 to prevent signaling. This is important to promote bacterial survival but also kidney pathology. Decoy adapters had been reported previously in Vaccinia virus (Bowie et al., 2000; Harte et al., 2003; Stack et al., 2005) and would therefore appear to be a common strategy for pathogens to evade innate immune responses. Several other bacterial species have TIR domain-containing proteins (Cirl et al., 2008), and this is likely to be a very fruitful area of research for those interested in virulence.

There have also been reports on single-nucleotide polymorphisms in the adapters that regulate their function, the best example so far being the S180L variant in Mal (Hawn et al., 2006; Khor et al., 2007; Castiblanco et al., 2008). The leucine

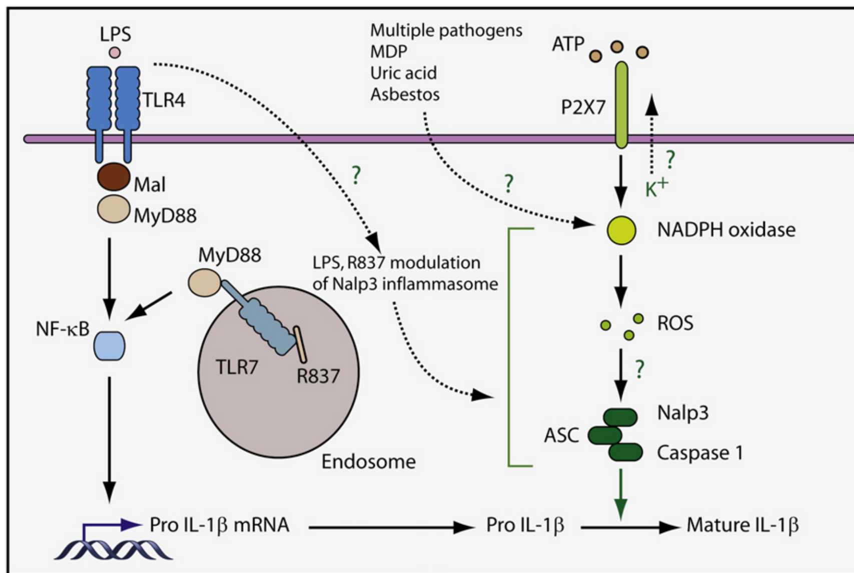

Figure 1. Crosstalk between TLRs and Nalp3

The induction of mature IL-1β is a complex interplay between TLRs and Nalp3. TLRs such as TLR4 and TLR7 activate the standard signaling pathway via MyD88 and NF-κB to induce pro-IL-1β mRNA. Various stimuli act via Nalp3 to activate caspase-1, which processes the pro-IL-1β protein into mature IL-1β. These stimuli include ATP, MDP, uric acid, and asbestos. A potassium efflux appears to be required, although how this occurs is still not fully worked out, nor is its role in Nalp3 activation. Uric acid and asbestos have been shown to activate NADPH-oxidase-generating reactive oxygen species (ROS), which are also required for Nalp3 activation, although again a mechanism is missing. Finally, LPS and R837 prime the Nalp3 inflammasome for activation and, intriguingly, this appears to be TLR independent.

form of Mal is fundamentally impaired and may act to temper signaling by the serine form. Heterozygotes are protected against multiple infectious disease, including malaria and TB (Khor et al., 2007; Castiblanco et al., 2008), and also in systemic autoimmune disease such as systemic lupus erythematosus (SLE) (Castiblanco et al., 2008). These studies confirm the importance of TLR signaling in human disease.

Crosstalk with Other Innate Receptors

The first example of where there has been progress regarding crosstalk is with other receptors involved in innate immunity. Because it is highly unlikely that the host will be exposed to single ligands for innate receptors during infection, a more physiological although challenging approach is to examine activation of signaling by multiple ligands simultaneously. The most studied combination so far is to examine TLR and NLR ligands. A clear-cut example of this "two-signal" approach is the ability of TLRs to induce pro-IL-1β production, but without causing processing to the mature cytokine, as shown in Figure 1. If caspase-1 is active, however, this processing will occur (Martinon, 2008). Nalp3 is a key component of an important caspase-1-containing inflammasome and is activated by various pathogens; such substances as ATP, MDP (Martinon et al., 2004); uric acid crystals (Martinon et al., 2006); and, most recently, other types of crystals such as asbestos and silica (Dostert et al., 2008). The mechanism of Nalp3 activation has been shown to involve a potassium efflux, mediated by the P2X7 receptor in combination with the ion channel pannexin-1 (Kanneganti et al., 2007a). It also appears to involve reactive oxygen species (ROS) generated by the NADPH oxidase, although how the potassium efflux or ROS generation leads to Nalp3 activation is not known. TLR ligands such as LPS or the TLR7 ligand R837 also modulate the inflammasome, enabling ATP to activate caspase-1 via Nalp3. Intriguingly this phenomenon appears to be independent of TLRs because it occurs in MyD88-, Mal-, and Trif-deficient macrophages and, in the case of R837, in TLR7-deficient macrophages (Yamamoto et al., 2004; Kanneganti et al., 2006; Mariathasan et al., 2006; Miggin et al., 2007) (Figure 1).

How ligands such as ATP, MDP, and uric acid crystals in this context are sensed is therefore unclear. It could be via induction of inflammasome components, although given the lack of a role for TLRs, this seems unlikely. It is also possible that the effect here is via modulation of ROS in some way, possibly via effects on the NADPH oxidase. Caspase-1 has also been shown to be constitutively active in monocytes, unlike macrophages, where it requires activation (Netea et al., 2008). This is likely to be due to monocytes being a "front-line" blood cell and will release mature IL-1β after induction of the proform.

Similarly synergies occur between TLRs and NOD1 or NOD2 (Tada et al., 2005; van Heel et al., 2005; Kanneganti et al., 2007b). TLRs and NOD1 or NOD2 have been shown to signal to NF-κB via the protein kinase RICK, which could be a point of synergy (Kobayashi et al., 2002). This is somewhat controversial, however, because there have also been reports that NOD2 ligands such as MDP inhibit signaling by multiple TLRs. This inhibitory effect was shown to involve IRF4 (Watanabe et al., 2008). In addition, Nod1- and Nod2-mediated signaling has been shown to be enhanced in macrophages made tolerant to TLR activation (Kim et al., 2008). This could be a fail-safe mechanism to allow host defense responses to intracellular pathogens. More mechanistic work is needed on these combinatorial experiments to provide clearer insights.

A more straightforward synergy has been reported between the CLR Dectin-1 and TLRs (Dennehy et al., 2008). Dectin-1 is essential for the innate response to fungal pathogens (Brown et al., 2002; Taylor et al., 2007). Its ligand is beta-1,3-glucan, and it signals via the tyrosine kinase Syk and the protein CARD9 (Hara et al., 2007) Clear synergies between Dectin-1 and TLR2 have been reported, although the fungal ligand for TLR2 awaits definition. Syk is absolutely required for this synergy, as is MyD88 (Dennehy et al., 2008). The role for Syk here contrasts with the inhibitory effect of DAP12 described below, because DAP12 has also been shown to require Syk to signal. The basis for this difference is not known, although the downstream signals activated by Syk in both cases might differ. As with the other synergies discussed here, we await a detailed mechanistic explanation.

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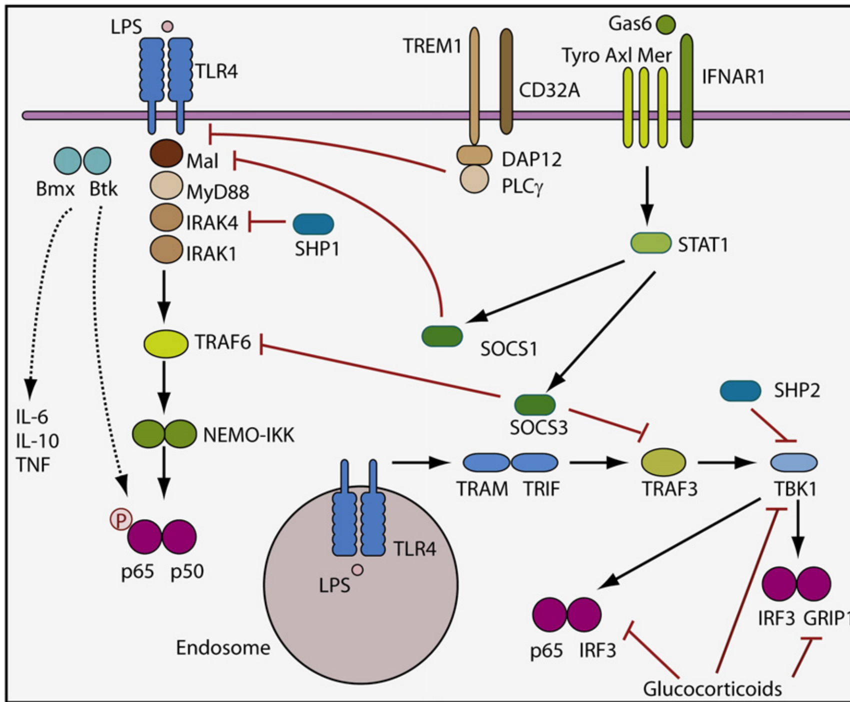

Figure 2. Crosstalk among TLRs, Tyrosine Kinases, Tyrosine Phosphatases, and Glucocorticoids

Multiple signaling pathways interact with TLR4 signals. Bruton's tyrosine kinase and in certain cell types Bmx are required for TLR4 signaling. Btk is on a pathway that leads to the phosohorylation of p65 on serine 536. It is also required for induction of IL10, IL6, and TNF. TREM1 and DAP12, probably acting via phospholipase C-gamma, limits TLR4 signaling. TAM receptors in a complex with IFNAR1 induce SOCS1, which inhibits TLR4 by targeting Mal, and SOCS3, which inhibits TRAF3 and TRAF6. TLR4 in endosomes recruits TRAM and TRIF, leading to TRAF3 activation. This pathway leads to TBK1 activation, which is required for IRF3 phosphorylation. The tyrosine phosphatase SHP1 inhibits IRAK1, limiting NF-κB, but in addition, this somehow boosts interferon-β production (not shown). SHP2, in contrast, inhibits TBK1 activation, blocking this response. Finally, glucocorticoids target the IRF3 pathway at multiple points—inhibition of a p65-IRF3 complex, disruption of the transactivator GRIP1, and inhibition of TBK1.

Crosstalk Via Tyrosine Kinases and Phosphatases

The second example of crosstalk recently characterized concerns the regulation of TLR signaling by tyrosine kinases and phosphatases (Figure 2). The role of tyrosine kinases in TLR signaling has been explored for a number of years, particularly in TLR4 signaling. LPS had been shown to increase tyrosine phosphorylation in cells, and various candidate tyrosine kinases have been suggested to participate in this process. Notably, the tyrosine kinases Src, Hck, and Lyn have been shown to be activated by TLR4, although their exact role is uncertain because a hck, fyn, lyn triple-deficient mouse was normal for TLR4 signaling (Meng and Lowell, 1997). More recently, Tec-family tyrosine kinases have been analyzed, and a role for Bruton's tyrosine kinase (Btk) in TLR4 signaling (and other TLRs such as TLR2, TLR4, TLR7-TLR8, and TLR9 [Jefferies et al., 2003; Horwood et al., 2003; Liljeroos et al., 2007; Doyle et al., 2007; Lee et al., 2008]) has been indicated. Btk is recruited to both TLR4 and TLR9 and has been shown to be involved in the pathway leading to enhanced phosphorylation of the p65 subunit of NF-κB on serine 536, which is required for p65 to promote gene expression (Doyle et al., 2005, 2007; Lee et al., 2008). In B cells, Btk is required for TLR9-induced proliferation and IL-10 production (Lee et al., 2008). Btk is also required for IL-10 induction by TLR4 in macrophages (Schmidt et al., 2006). These effects on IL-10 can confound cytokine readouts because in Btk-deficient cells there have been reports of enhanced IL-12 and IL-6 production, which is due to the decrease in IL-10, leading to a loss of autocrine inhibition of these cytokines. There are inconsistencies in the literature, however, because in human peripheral blood mononuclear cells there appears to be no role for Btk in IL-6 induction in response to TLR2 or TLR4 (Horwood et al., 2006). There is a role in TNF production, however, which was shown to be due to Btk's being required for p38 activation, which then stabilizes TNF but not IL-6 mRNA. Another Tec family member, Bmx, has been shown to be required for TLR4-induced IL6 production in macrophages and rheumatoid arthritis synovial cells (Palmer et al., 2008a, 2008b), via mRNA stabilization, although the mechanism is not known.

Most notably, cells from patients with X-linked agammaglobulinemia, which harbor a mutation in Btk, are defective in their capacity to synthesize cytokines in response to TLR2, TLR4, TLR7-TLR8, and TLR9 (Doyle et al., 2007; Taneichi et al., 2008). The precise downstream targets for Btk are, however, still unclear. One substrate is Mal (Gray et al., 2006; Piao et al., 2008), which undergoes phosphorylation by Btk on Tyr-86 and Tyr-106. This event is required for Mal to signal NF-κB activation but, in addition, is involved in the ultimate degradation of Mal via the recruitment of suppressor of cytokine signaling 1 (SOCS1) (Mansell et al., 2006). This appears to be an important aspect of SOCS1—mediated inhibition of TLR4 signaling. This phosphorylation of Mal has also been shown to be impaired in cells rendered tolerant to LPS (Piao et al., 2008).

An inhibitory role for proteins with an immunoreceptor tyrosine-based activation motif (ITAM) toward TLR signaling has also been suggested (Hamerman et al., 2005). At first, this seemed counterintuitive, because ITAM-containing proteins that couple to receptors had been shown to activate signaling pathways leading to inflammatory cytokine production, such as DAP12, which couples to the coreceptor TREM-1. The tyrosine kinase Syk plays a key role in DAP12 activation, phosphorylating tyrosine residues, and allowing for the recruitment of SH2-domain-containing proteins such as the p85-p110 dimer of PI3 kinase and phospholipase Cγ. TREM-1 had been shown to synergize with TLR4 in the induction of proinflammatory cytokines (Bouchon et al., 2001). However, DAP12-deficient mice were shown to be more responsive to TLR stimulation,

producing higher quantities of cytokines in response to various TLR ligands (Hamerman et al., 2005). It therefore appears that DAP12-associated receptors inhibit TLR signaling by an unknown mechanism. One interesting aspect of this area is a recent paper demonstrating that CD32A (a so-called ''activating'' Fcγ receptor), which has an ITAM, inhibits TLR signaling (Dunn-Siegrist et al., 2007). This was shown through the use of an antibody to TLR4, whose inhibitory effect was shown to be dependent on Fcγ receptor signaling. The effect here appears to be proximity dependent—the antibody binds to TLR4 and the Fc portion binds to CD32A, bringing it into close proximity to TLR4. How it inhibits TLR4 is not clear, however. It might involve activation of PI3 kinase, which via Akt has been shown to block NF-κB activation by TLR4. Alternatively, because CD32A can activate phospholipase C-γ, a depletion in PIP2 by this enzyme might lead to Mal's dissociation from the plasma membrane. Depletion in PIP2 would therefore be expected to limit TLR4 signaling. This capacity of Fc receptors to inhibit TLR4 might be physiologically relevant because it is possible that once adaptive immunity is established, limiting innate immunity via Fc receptors might protect the host from deleterious effects of inflammation and sepsis.

Further evidence of tyrosine kinases inhibiting TLR signaling has come from studies into the TAM family of receptor tyrosine kinases (Rothlin et al., 2007). When originally characterized, the TAM receptors were orphans. Two agonists were then found—growth-arrest-specific 6 (Gas6) and protein S (Stitt et al., 1995). Gas6 binds all three, although with different affinities. Protein S may be specific for Tyro. A triple-deficient mouse was generated in order to determine the function of these receptors. These mice had a clear immune phenotype—they developed a systemic autoimmune disease. The basis for this appeared to be a lack of inhibition of signaling by TLRs in the mice. The mechanism was a failure to induce SOCS1 and SOCS3, proteins that inhibit JAK-STAT signaling pathways, as well as TLR signaling (Rothlin et al., 2007). The ligands for the TAM receptors Gas6 and Protein S activate a signaling pathway via the R1 subunit of the type I interferon receptor, which has been shown to occur in a complex with Axl. This leads to activation of STAT1 and, in turn, induction of SOCS1 and SOCS3, which act to block TLR signaling. If mice are deficient in TAM receptors, this pathway doesn't occur and TLR signaling is potentiated. That autoimmunity results tells us that altered innate immunity is sufficient to promote dysfunctional B and T lymphocyte activation.

Tyrosine phosphatases can negatively regulate certain TLR responses. SH2-containing protein tyrosine phosphatase 1 (SHP1) is an intracellular tyrosine phosphatase that contains two tandemly linked SH2 domains at its amino terminus, followed by a catalytic domain. It has been shown that SHP1 negatively regulates TLR-mediated production of proinflammatory cytokines by inhibiting activation of NF-κB and MAP kinases (An et al., 2008). Intriguingly, SHP1 promotes production of type I interferons by TLRs and also RIG-I. The mechanism in both cases appears to be SHP1 binding to the kinase domain of IRAK-1 (which contains several phosphoaccepting tyrosines), where it inhibits its activity. This kinase activity is required for induction of proinflammatory cytokines, but is somehow inhibitory for induction of type I interferons, because autophosphoylation of IRAK1 leads to its degradation, allowing for increased production of type I interferons (An et al., 2008). IRAK1 may be acting as a bridging adaptor for the pathway to type I interferon production, although there is conflicting evidence regarding its action as a kinase to phosphorylate IRF7, which is required for type I interferon gene transcription (Uematsu et al., 2005).

The related SHP, SHP2, also negatively regulates TLR signaling, but its effect is more specific (An et al., 2006). SHP2 limits TLR3-induced production of proinflammatory cytokines and interferon-β, without affecting TLR2 or TLR9 signaling. In this case, SHP2 was shown to target TBK-1, the kinase that phosphorylates IRF3. Although related, therefore, SHP1 and SHP2 have opposite effects. SHP1 promotes interferon-β production in response to TLRs, whereas SHP2 is inhibitory. Clearly, the overall mechanism here is for each SHP to bind to phosphotyrosine residues on specific substrates, limiting the function of the target. In the case of SHP1 this is IRAK1, whereas for SHP2 it is TBK-1. How this binding translates into an inhibitory effect is still unclear.

Regulation via Ubiquination

The third example of crosstalk concerns regulation of TLR signaling by ubiquitination. An extensive literature on this subject has been building up recently. Various ubiquitin ligases have been implicated, and in fact some of the components found in TLR signaling turn out to be ubiquitin ligases themselves. The recent literature is allowing us to establish the earliest catalytic events in TLR signaling, which involve both phosphorylation and ubiquitination of components, leading to their activation and/or degradation (Figure 3). As described above, TLR signaling (with the exception of TLR3) is initiated by the recruitment of MyD88 to the TLR complex. This in turn leads to the recruitment of IRAK1 and IRAK4. IRAK4 then activates IRAK1, allowing IRAK1 to autophosphorylate. Both IRAK1 and IRAK4 then dissociate from MyD88, which remains in a complex with the activated receptor—this has been shown for IL-1RI and will most probably apply to the TLRs (Brikos et al., 2007). They in turn interact with TRAF6, which is an E3 ubiquitin ligase. TRAF6 is then thought to autoubiquinate, attaching K63-polyubiquitin to itself. The E2-conjugating complexes Ubc13 and Uev1a are also involved in this process (Deng et al., 2000). K63-polyubiquitin-TRAF6 can then recruit TAK1 in a complex with TAB2 and TAB3, which both contain nuclear zinc-finger motifs that interact with K63-polyubiquitin chains. This somehow activates TAK1, which then couples to the IKK complex, which contains the scaffold protein NEMO and the kinase responsible for phosphorylation of I-kappaB, IKK2. TAK1 also couples to MKK3-MKK6 and MKK7, the respective upstream kinases for p38 and JNK.

The situation regarding the initiation of TLR signaling is likely to be more complex, however. It has been shown that another member of the IRAK family, IRAK2, appears to be more important than IRAK1 in the pathway to Traf6 ubiquitination. This is particularly the case for TLR3, which doesn't signal via IRAK1 or IRAK4 (Keating et al., 2007). The IRAK2-deficient mouse has also recently been described (Kawagoe et al., 2008). It was shown that similar to IRAK1, IRAK2 was activated downstream of IRAK4. Also similar to IRAK1, IRAK2 is essential for signaling because TLR-induced cytokine production was abrogated in the absence IRAK2. Importantly, the role of IRAK2 was in the sustained activation of NF-κB. Finally, IRAK2 was shown to possess kinase activity. This was thought to be unlikely

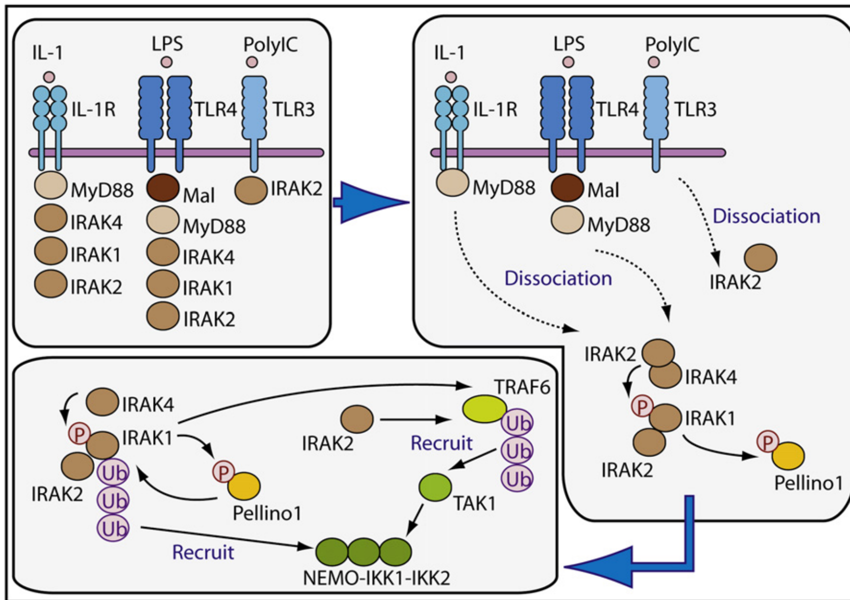

Figure 3. Four Steps to NF-κB Activation by TLRs

A clearer picture of how TLRs lead to activation of NF-κB activation has emerged. Phosphorylation and ubiquitination are key covalent modifications in this process. Signaling is initiated by ligand binding, shown here for IL-1, LPS, and pIC. In IL-1 signaling, MyD88 is recruited to the receptor complex within seconds, where it stably associates. For TLR4, Mal is required to recruit MyD88. IRAK4 and IRAK1 (and probably IRAK2) are then recruited to MyD88. For TLR3, IRAK2 is directly recruited. IRAK4 then phosphorylates IRAK1, and the kinases dissociate from MyD88. IRAK1 phosphorylates Pellino-1. IRAK2 probably dissociates from TLR3, although the mechanism is not known. Pellino-1 is an E3-ligase that causes K63-polyubiquination of IRAK1. This serves as a platform to recruit the NEMO-IKK1-IKK2 complex. IRAK2 leads to K63-linked polyubiquitination of TRAF6, which serves as a platform to recruit TAK1. TAK1 then activates IKK2 in the NEMO-IKK1-IKK2 complex. IRAK1 has a similar role to IRAK2—it leads to K63-linked polyubiquitination of TRAF6 and TAK1 recruitment. This brings TAK1 into close proximity with IKK2 in the NEMO-IKK1-IKK2 complex, activating IKK2. How NEMO-IKK1-IKK2 is brought into proximity with TAK1 in the IRAK2 pathway is not known. IRAK2 has also recently been shown to be important for the sustained activation of NF-κB downstream of IRAK4 and IRAK1, with the possible exception of TLR3.

because it was predicted to be a pseudokinase. Consistent with its role in a sustained response, IRAK2 kinase activity was sustained and peaked at 8 hr after stimulation, unlike IRAK1, which peaked at 1 hr stimulation. It therefore appears that IRAK1 and IRAK2 act in sequence, with both being essential for cytokine induction by TLR2. No role for IRAK2 was found in TLR3 signaling, however, because this was normal in the IRAK2-deficient mice.

There is also new complexity in our understanding of IRAK1 function. In response to IL-1 (and, by analogy, presumably the TLRs, given that the IL-1 type I receptor signals via MyD88), IRAK1 undergoes K63-linked polyubiquination. Two proteins, termed Pellino-1 and Pellino-3b, appear to be important in this process (Butler et al., 2007; Ordureau et al., 2008). These proteins were known to interact with IRAK-1, and recent studies indicate that similar to Traf6, they are E3 ligases. Importantly, their activity is greatly enhanced, at least in vitro, in response to phosphorylation by IRAK1 and IRAK4. The role of the kinase activity of IRAK1 and/or IRAK4 might therefore be at least initially to activate Pellinos, such that they can cause K63-linked polyubiquitination of IRAK1. Most interestingly, this recruits NEMO to IRAK1, with NEMO binding to polyubiquitin. There is also evidence that TRAF6 ubiquitinates IRAK1 (Conze et al., 2008). It therefore appears that the mechanism to activate the IKK complex involves recruiting the complex to IRAK1. This may be the mechanism to bring TAK1 into close proximity with the IKKs to allow for their activation. These studies therefore for the first time allow us to kinetically map the covalent modifications that lead to assembly of the complex that triggers NF-κB activation, with both phosphorylation and ubiquitination playing key roles (Figure 3). Ultimately, both IRAK1 and the Pellinos (which also undergo ubiquitination) are probably degraded to terminate the signal.

The importance of ubiquitination for TLR signaling in vivo has been emphasized in two studies. A20 is a key ubiquitin-editing enzyme that restricts TLR responses by regulating the ubiquitination of key signaling proteins such as Traf6. A20-deficient mice have a severe phenotype, with multiorgan inflammation and premature death. Crossing these mice with MyD88-deficient mice rescues this phenotype (Turer et al., 2008). This is because the phenotype is due to TLR activation by commensal bacteria. A20 is therefore key to keeping overactivation of TLRs in check, by targeting K63-linked polyubiquination of proteins such as Traf6. In the second study, a protein termed TRIM30-alpha, which has a RING finger (often indicative of E3 ubiquitin ligase activity) was shown to limit TLR signaling by targeting TAB2 and TAB3, causing their degradation. This effect was not via the ubiquitin-proteasome pathway, however, but depended on lysosomal degradation (Shi et al., 2008). This study points to the importance of TAB2 and TAB3 (and, by association, TAK1) in TLR signaling and emphasizes gaps in our knowledge of the regulation of TLR signaling by ubiquitination.

Crosstalk with Glucocorticoids

Finally, work on the effects of glucocorticoids on TLR signaling have revealed complex crosstalk mechanisms to explain their anti-inflammatory effects. Multiple targets for glucocorticoids have been reported over the years, notably effects on the NF-κB pathway (Clark, 2007). Recently, it has been shown that glucocorticoids can inhibit activation of p38 MAP kinase by TLRs. The mechanism was shown to involve induction of a phosphatase termed MKP-1 (Lasa et al., 2002; Abraham et al., 2006; Zhao et al., 2006), which dephosphorylates p38 and thereby inactivates it. It was shown that MKP-1-deficient mice are less susceptible to the inhibitory effects of glucocorticoids, pointing

to the importance of this process for the effects of glucocorticoids in vivo (Abraham et al., 2006). Another study demonstrated that glucocorticoids appear especially potent at inhibiting the induction of IRF3-dependent genes by TLR4 but not TLR3 (Ogawa et al., 2005). This apparent specificity was shown to be due to the targeting of a p65-IRF3 complex, which had been shown to be activated by TLR4 but not TLR3 (Wietek et al., 2003). However, two other studies indicate that the effect of glucocorticoids may not be restricted to TLR4 relative to TLR3. GRIP1 is a protein that interacts with the glucocorticoid receptor. A yeast two-hybrid screen isolated IRF3 as an interacting protein, and it was shown that GRIP1 was required for IRF3-mediated transactivation. Importantly the glucocorticoid receptor competed with IRF3 for GRIP1 and thereby blocked IRF3-mediated gene expression (Reily et al., 2006). This was the case for both TLR3 and TLR4. In addition, another study has shown that activation of TBK-1, the upstream kinase for IRF3, is inhibited by glucocorticoids (McCoy et al., 2008), again pointing to a more general mechanism rather than inhibition of TLR4 specifically. It may be that for lower doses of glucocorticoids, there is a tendency toward TLR4 specificity, but this would have to be tested systematically.

Conclusions

Signaling pathways activated by TLRs continue to reveal novelty and complexity. Additional components and regulation of known components continue to be uncovered. Importantly, several other signaling systems have an impact on TLR signaling, notably signals activated by other innate receptors, tyrosine kinases and tyrosine phosphatases, ubiquitinating systems, and glucocorticoids. These interactions will obviously be context dependent and may be cell-type specific. However, the characterization of these interactions is an important goal, because it will provide us with models to explain TLR signaling in vivo, during infection and inflammation.

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REFERENCES

Abraham, S.M., Lawrence, T., Kleiman, A., Warden, P., Medghalchi, M., Tuckermann, J., Saklatvala, J., and Clark, A.R. (2006). Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. J. Exp. Med. *203*, 1883–1889.

An, H., Zhao, W., Hou, J., Zhang, Y., Xie, Y., Zheng, Y., Xu, H., Qian, C., Zhou, J., Yu, Y., et al. (2006). SHP-2 phosphatase negatively regulates the Trif adaptor protein-dependent Type I interferon and proinflammatory cytokine production. Immunity *25*, 919–928.

An, H., Hou, J., Zhou, J., Zhao, W., Xu, H., Zheng, Y., Yu, Y., Liu, S., and Cao, X. (2008). Phosphatase SHP-1 promotes TLR- and RIG-I-activated production of type I interferon by inhibiting the kinase IRAK1. Nat. Immunol. *9*, 542–550.

Bouchon, A., Facchetti, F., Weigand, M.A., and Colonna, M. (2001). TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature *410*, 1103–1107.

Bowie, A., Kiss-Toth, E., Symons, J.A., Smith, G.L., Dower, S.K., and O'Neill, L.A. (2000). A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. Proc. Natl. Acad. Sci. USA *97*, 10162–10167.

Bowie, A.G., and O'Neill, L.A.J. (2007). The family of five: The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat. Rev. Immunol. *7*, 353–364.

Brikos, C., Wait, R., Begum, S., O'Neill, L.A., and Saklatvala, J. (2007). Mass spectrometric analysis of the endogenous IL-1RI signalling complex formed after IL-1 binding, identifies IL-1RAcP, MyD88 and IRAK-4 as the stable components. Mol. Cell Proteomics *6*, 1551–1559.

Brown, G.D., Taylor, P.R., Reid, D.M., Willment, J.A., Williams, D.L., Martinez-Pomares, L., Wong, S.Y., and Gordon, S. (2002). Dectin-1 is a major beta-glucan receptor on macrophages. J. Exp. Med. *196*, 407–412.

Butler, M.P., Hanly, J.A., and Moynagh, P.N. (2007). Kinase active IRAKs promote polyubiquination and degradation of the pellino family: direct evidence for pellino proteins being E3 ubiquitin ligases. J. Biol. Chem. *282*, 29729–29737.

Carty, M., Goodbody, R., Schröder, M., Stack, J., Moynagh, P.N., and Bowie, A.G. (2006). The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. Nat. Immunol. *7*, 1074–1081.

Castiblanco, J., Varela, D.C., Castaño-Rodríguez, N., Rojas-Villarraga, A., Hincapié, M.E., and Anaya, J.M. (2008). TIRAP (MAL) S180L polymorphism is a common protective factor against developing tuberculosis and systemic lupus erythematosus. Infect. Genet. Evol., in press. Published online March 12, 2008. 10.1016/j.meegid.2008.03.001.

Cirl, C., Wieser, A., Yadav, M., Duerr, S., Schubert, S., Fischer, H., Stappert, D., Wantia, N., Rodriguez, N., Wagner, H., et al. (2008). Nat. Med. *14*, 399–406.

Clark, A.R. (2007). Anti-inflammatory functions of glucocorticoid genes. Mol. Cell. Endocrinol. *275*, 79–97.

Conze, D.B., Wu, C.J., Thomas, J.A., Landstrom, A., and Ashwell, J.D. (2008). Lys (K)63-linked polyubiquitination of IRAK-1 is required for IL-1 receptor- and Toll-like receptor-mediated NF-kappaB activation. Mol. Cell. Biol., in press. Published online March 17, 2008. 10.1128/MCB.02098-07.

Creagh, E.M., and O'Neill, L.A.J. (2006). TLRs, NLRs and RLRs: A trinity of pathogen sensors that co-operate in innate immunity. Trends Immunol. *27*, 352–357.

Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C., and Chen, Z.J. (2000). Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell *103*, 351–361.

Dennehy, K.M., Ferwerda, G., Faro-Trinidade, I., Pyz, E., Willment, J.A., Taylor, P.R., Kerrigan, A., Tsoni, V., Gordon, S., Meyer-Wentrup, F., et al. (2008). Syk kinase is required for collaborative cytokine production induced through Dectin-1 and Toll-like receptors. Eur. J. Immunol. *38*, 500–506.

Dostert, C., Pétrilli, V., Van Bruggen, R., Steele, C., Mossman, B.T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science *320*, 674–677.

Doyle, S.L., Jefferies, C.A., and O'Neill, L.A. (2005). Bruton's tyrosine kinase is involved in p65-mediated transactivation and phosphorylation of p65 on serine 536 during NF-kappa B activation by LPS. J. Biol. Chem. *280*, 23496–23501.

Doyle, S.L., Jefferies, C.A., Feighery, C., and O'Neill, L.A. (2007). Signaling by Toll-like receptors 8 and 9 requires Bruton's tyrosine kinase. J. Biol. Chem. *282*, 36953–36960.

Dunn-Siegrist, I., Leger, O., Daubeuf, B., Poitevin, Y., Depis, F., Herren, S., Kosco-Vilbois, M., Dean, Y., Pugin, J., and Elson, G. (2007). Pivotal involvement of Fc-gamma receptor IIA in the neutralisation of lipopolysaccharide signalling via a potent novel anti-TLR4 monoclonal antibody 15C1. J. Biol. Chem. *282*, 34817–34827.

Gray, P., Dunne, A., Brikos, C., Jefferies, C.A., Doyle, S.L., and O'Neill, L.A. (2006). MyD88 Adapter-like (Mal) is phosphorylated by Bruton's tyrosine kinase during TLR2 and TLR4 signal transduction. J. Biol. Chem. *281*, 10489–10495.

Hamerman, J.A., Tchao, N.K., Lowell, C.A., and Lanier, L.L. (2005). Enhanced Toll-like receptor responses in the absence of signalling adaptor DAP12. Nat. Immunol. *6*, 579–586.

Hara, H., Ishihara, C., Takeuchi, A., Imanishi, T., Xue, L., Morris, S.W., Inui, M., Takai, T., Shibuya, A., Saijo, S., et al. (2007). The adaptor protein CARD9 is

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essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors. Nat. Immunol. *8*, 619–629.

Harte, M.T., Haga, I.R., Maloney, G., Gray, P., Reading, P.C., Bartlett, N.W., Smith, G.L., Bowie, A., and O'Neill, L.A. (2003). The poxvirus protein A52R targets Toll-like receptor signaling complexes to suppress host defense. J. Exp. Med. *197*, 343–351.

Hawn, T.R., Dunstan, S.J., Thwaites, G.E., Simmons, C.P., Thuong, N.T., Lan, N.T., Quy, H.T., Chau, T.T., Hieu, N.T., Rodrigues, S., et al. (2006). Polymorphism in Toll-interleukin 1 receptor domain containing adaptor protein is associated with susceptibility to meningeal tuberculosis. J. Infect. Dis. *194*, 1127–1134.

Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., and Yoshida, N. (2005). Taniguchi T.IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature *434*, 772–777.

Horwood, N.J., Mahon, T., McDaid, J.P., Campbell, J., Mano, H., Brennan, F.M., Webster, D., and Foxwell, B.M. (2003). Bruton's tyrosine kinase is required for lipopolysaccharide-induced tumor necrosis factor alpha production. J. Exp. Med. *197*, 1603–1611.

Horwood, N.J., Page, T.H., McDaid, J.P., Palmer, C.D., Campbell, J., Mahon, T., Brennan, F.M., Webster, D., and Foxwell, B.M. (2006). Bruton's tyrosine kinase is required for TLR2 and TLR4-induced TNF production, but not IL6 production. J. Immunol. *176*, 3635–3641.

Jefferies, C.A., Doyle, S., Brunner, C., Dunne, A., Brint, E., Wietek, C., Walch, E., Wirth, T., and O'Neill, L.A. (2003). Bruton's tyrosine kinase is a TIR domain binding protein that participates in NFkappa B activation by Toll-like receptor 4. J. Biol. Chem. *278*, 26258–26264.

Kagan, J.C., and Medzhitov, R. (2006). Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling. Cell *125*, 943–955.

Kagan, J.C., Su, T., Horng, T., Chow, A., Akira, S., and Medzhitov, R. (2008). Tram couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. Nat. Immunol. *9*, 361–368.

Kanneganti, T.D., Lamkanfi, M., Kim, Y.G., Chen, G., Park, J.H., Franchi, L., Vandenabeele, P., and Núñez, G. (2007a). Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. Immunity *26*, 433–443.

Kanneganti, T.D., Lamkanfi, M., and Nunez, G. (2007b). Intracellular NOD-like receptors in host defense and disease. Immunity *27*, 549–559.

Kanneganti, T.D., Özören, N., Body-Malapel, M., Am, A., Park, J.H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., et al. (2006). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature *440*, 233–236.

Kawagoe, T., Sato, S., Matsushita, K., Kato, H., Matsui, K., Kumagai, Y., Saitoh, T., Kawai, T., Takeuchi, O., and Akira, S. (2008). Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. Nat. Immunol. *9*, 684–691.

Keating, S.E., Maloney, G.M., Morna, E.M., and Bowie, A.G. (2007). IRAK-2 participates in multiple Toll-like receptor signaling pathways to NF-kappaB via activation of Traf6 ubiquitination. J. Biol. Chem. *282*, 33435–33443.

Khor, C.C., Chapman, S.J., Vannberg, F.O., Dunne, A., Murphy, C., Ling, E.Y., Frodsham, A.J., Walley, A., Kyrieleis, O., Khan, A., et al. (2007). A functional variant in MAL/TIRAP and protection against invasive pneumococcal disease, bacteraemia, malaria and tuberculosis. Nat. Genet. *39*, 523–528.

Kim, Y., Zhou, P., Qian, L., Chuang, J.Z., Lee, J., Li, C., Iadecola, C., Nathan, C., and Ding, A. (2007). MyD88–5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. J. Exp. Med. *204*, 2063–2074.

Kim, Y.G., Park, J.H., Shaw, M.H., Franchi, L., Inohara, N., and Nunez, G. (2008). The cytosolic sensors Nod1 and Nod2 are critical for bacterial recognition and host defense after exposure to Toll-like receptor ligands. Immunity *28*, 246–257.

Kobayashi, K., Inohara, N., Hernandez, L.D., Galán, J.E., Núñez, G., Janeway, C.A., Medzhitov, R., and Flavell, R.A. (2002). RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature *416*, 194–199.

Lasa, M., Abraham, S.M., Boucheron, C., Saklatvala, J., and Clark, A.R. (2002). Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. Mol. Cell. Biol. *22*, 7802–7811.

Latz, E., Verma, A., Visintin, A., Gong, M., Sirois, C.M., Klein, D.C., Monks, B.G., McKnight, C.J., Lamphier, M.S., Duprex, W.P., et al. (2007). Ligand-induced conformational changes allosterically activate Toll-like receptor 9. Nat. Immunol. *8*, 772–779.

Lee, K.G., Xu, S., Wong, E.T., Tergaonkar, V., and Lam, K.P. (2008). Bruton's tyrosine kinase separately regulates NF-kappaB p65relA activation and cytokine interleukin (IL)-10/IL-12 production in TLR9-stimulated B cells. J. Biol. Chem. *283*, 11189–11198.

Liljeroos, M., Vuolteenaho, R., Morath, S., Hartung, T., Hallman, M., and Ojaniemi, M. (2007). Bruton's tyrosine kinase together with PI 3-kinase are part of Toll-like receptor 2 multiprotein complex and mediate LTA induced Toll-like receptor 2 responses in macrophages. Cell. Signal. *19*, 625–633.

Mansell, A., Simth, R., Doyle, S., Gray, P., Fennen, J.E., Crack, P., Nicholson, S., Hilton, D.J., O'Neill, L.A., and Hertzog, P. (2006). Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by targeting Mal for degradation. Nat. Immunol. *7*, 148–155.

Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W.P., Weinrauch, Y., Monack, D.M., and Dixit, V.M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. Nature *440*, 228–232.

Martinon, F. (2008). Detection of immune danger signals by NALP3. J. Leukoc. Biol. *83*, 507–511.

Martinon, F., Pétrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature *440*, 237–241.

Martinon, F., Agostini, L., Meylan, E., and Tschopp, J. (2004). Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. Curr. Biol. *14*, 1929–1934.

McCoy, C.E., Carpenter, S., Palsson-McDermott, E.M., Gearing, L.J., and O'Neill, L.A. (2008). Glucocorticoids inhibit IRF3 phosphorylation in response to Toll-like receptor-3 and -4 by targeting TBK1 activation. J. Biol. Chem. *283*, 14277–14285.

McGettrick, A., Brint, E.K., Palsson-McDermott, E.M., Rowe, D.C., Golenbock, D.T., Gay, N.J., Fitzgerald, K.A., and O'Neill, L.A. (2006). Trif-related adapter molecule is phosphorylated by PKC{epsilon} during Toll-like receptor 4 signaling. Proc. Natl. Acad. Sci. USA *103*, 9196–9201.

Meng, F., and Lowell, C.A. (1997). Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn. J. Exp. Med. *185*, 1661–1670.

Miggin, S.M., Pålsson-McDermott, E., Dunne, A., Jefferies, C., Pinteaux, E., Banahan, K., Murphy, C., Moynagh, P., Yamamoto, M., Akira, S., et al. (2007). NF-kappaB activation by the Toll-IL-1 receptor domain protein MyD88 adapter-like is regulated by caspase-1. Proc. Natl. Acad. Sci. USA *104*, 3372–3377.

Netea, M.G., Nold, C., Nold, M., Opitz, B., van den Meer, J., Kullberg, B.J., Van den Meer, J., and Dinarello, C.A. (2008) Monocytes are not macrophages: One-hit fresus tow-hit stimulation of LPS-driven activation of the human inflammasome for IL-1beta. Abstract 265, Keystone Symposium: Innate Immunity: Signalling Mechanisms.

Newman, R.M., Salunkhe, P., Godzik, A., and Reed, J.C. (2006). Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. Infect. Immun. *74*, 594–601.

Núñez Miguel, R., Wong, J., Westoll, J.F., Brooks, H.J., O'Neill, L.A., Gay, N.J., Bryant, C.E., and Monie, T.P. (2007). A dimer of the Toll-like receptor 4 cytoplasmic domain provides a specific scaffold for the recruitment of signalling adaptor proteins. PLoS ONE. *2*, e788.

Ogawa, S., Lozach, J., Benner, C., Pascual, G., Tangirala, R.K., Westin, S., Hoffmann, A., Subramaniam, S., David, M., Rosenfeld, M.G., and Glass, C.K. (2005). Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. Cell *122*, 707–721.

Ordureau, A., Smith, H., Windheim, M., Peggie, M., Carrick, E., Morrice, N., and Cohen, P. (2008). The IRAK-catalysed activation of the E3 ligase function of Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1. Biochem. J. *409*, 43–52.

Palmer, C.D., Mutch, B.E., Page, T.H., Horwood, N.J., and Foxwell, B.M.J. (2008a). Bmx regulates LPS-induced IL-6 and VEGF production via mRNA stability in rheumatoid synovial fibroblasts. Biochem. Biophys. Res. Commun. *370*, 599–602.

Palmer, C.D., Mutch, B.E., Workman, S., McDaid, J.P., Horwood, N.J., and Foxwell, B.M.J. (2008b). Bmx tyrosine kinase regulates TLR4-induced IL-6 production in human macrophages independently of p38 MAPK and NF-kappaB activity. Blood *111*, 1781–1788.

Piao, W., Song, C., Chen, H., Wahl, L.M., Fitzgerald, K.A., O'Neill, L.A., and Medvedev, A.E. (2008). Tyrosine phosphorylation of MyD88 adapter-like (Mal) is critical for signal transduction and blocked in endotoxin tolerance. J. Biol. Chem. *282*, 3109–3119.

Reily, M.M., Pantoja, C., Hu, X., Chinenov, Y., and Rogatsky, I. (2006). The GRIP:IRF3 interaction as a target for glucocorticoid receptor-mediated immunosuppression. EMBO J. *25*, 108–117.

Rothlin, C.V., Ghosh, S., Zuniga, E.I., Oldstone, M.B.A., and Lemke, G. (2007). TAM receptors are pleiotropic inhibitors of the innate immune response. Cell *131*, 1124–1136.

Rowe, D.C., McGettrick, A.F., Latz, E., Monks, B.G., Gay, N.J., Yamamoto, M., Akira, S., O'Neill, L.A., Fitzgerald, K.A., and Golenbock, D.T. (2006). The myristoylation of TRIF-related adaptor molecule is essential for Toll-like receptor 4 signal transduction. Proc. Natl. Acad. Sci. USA *103*, 6299–6304.

Schmidt, N.W., Thieu, V.T., Mann, B.A., Ahyi, A.N., and Kaplan, M.H. (2006). Bruton's tyrosine kinase is required for TLR-induced IL-10 production. J. Immunol. *177*, 7203–7210.

Shi, M., Deng, W., Bi, E., Mao, K., Ji, Y., Lin, G., Wu, X., Tao, Z., Li, Z., Cai, X., et al. (2008). TRIM30 alpha negatively regulates TLR-mediated NF-kappa B activation by targeting TAB2 and TAB3 for degradation. Nat. Immunol. *9*, 369–377.

Stack, J., Haga, I.R., Schröder, M., Bartlett, N.W., Maloney, G., Reading, P.C., Fitzgerald, K.A., Smith, G.L., and Bowie, A.G. (2005). Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. J. Exp. Med. *201*, 1007–1018.

Stitt, T.N., Conn, G., Gore, M., Lai, C., Bruno, J., Radziejewski, C., Mattsson, K., Fisher, J., Gies, D.R., Jones, P.F., et al. (1995). The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell *80*, 661–670.

Tada, H., Aiba, S., Shibata, K., Ohteki, T., and Takada, H. (2005). Synergistic effect of Nod1 and Nod2 agonists with toll-like receptor agonists on human dendritic cells to generate interleukin-12 and T helper type 1 cells. Infect. Immun. *73*, 7967–7976.

Taneichi, H., Kanegane, H., Sira, M.M., Futatani, T., Agematsu, K., Sako, M., Kaneko, H., Kondo, N., Kaisho, T., and Miyawaki, T. (2008). Toll-like receptor signaling is impaired in dendritic cells from patients with X-linked agammaglobunemia. Clin. Immunol. *126*, 148–154.

Tanimura, N., Saitoh, S., Matsumoto, F., Akahi-Takamura, S., and Miyake, K. (2008). Roles for LPS-dependent interaction and relocation of TLR4 and Tram in Trif-signaling. Biochem. Biophys. Res. Commun. *368*, 94–99.

Taylor, P.R., Tsoni, S.V., Willment, J.A., Dennehy, K.M., Rosas, M., Findon, H., Haynes, K., Steele, C., Botto, M., Gordon, S., and Brown, G.D. (2007). Dectin-1 is required for beta-glucan recognition and control of fungal infection. Nat. Immunol. *8*, 31–38.

Turer, E.E., Tavares, R.M., Mortier, E., Hitotsumatsu, O., Advincula, R., Lee, B., Shifrin, N., Malynn, B.A., and Ma, A. (2008). Homeostatic MyD88-dependent signals cause lethal inflammation in the absence of A20. J. Exp. Med. *205*, 451–464.

Uematsu, S., Sato, S., Yamamoto, M., Hirotani, T., Kato, H., Takeshita, F., Matsuda, M., Coban, C., Ishii, K.J., Kawai, T., et al. (2005). Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-{alpha} induction. J. Exp. Med. *201*, 915–923.

van Heel, D.A., Ghosh, S., Butler, M., Hunt, K., Foxwell, B.M., Mengin-Lecreulx, D., and Playford, R.J. (2005). Synergistic enhancement of Toll-like receptor responses by NOD1 activation. Eur. J. Immunol. *35*, 2471–2476.

Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J., and Chen, Z.J. (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature *412*, 346–351.

Watanabe, T., Asano, N., Murray, P.J., Ozato, K., Tailor, P., Fuss, I.J., Kitani, A., and Strober, W. (2008). Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. J. Clin. Invest. *118*, 545–559.

Weber, A.N., Moncrieffe, M.C., Gangloff, M., Imler, J.L., and Gay, N.J. (2005). Ligand-receptor and receptor-receptor interactions act in concert to activate signaling in the Drosophila toll pathway. J. Biol. Chem. *280*, 22793–22799.

Wietek, C., Miggin, S.M., Jefferies, C.A., and O'Neill, L.A. (2003). Interferon regulatory factor-3-mediated activation of the interferon-sensitive response element by Toll-like receptor (TLR) 4 but not TLR3 requires the p65 subunit of NF-kappa. J. Biol. Chem. *278*, 50923–50931.

Willment, J.A., and Brown, G.D. (2008). C-type lectin receptors in antifungal immunity. Trends Microbiol. *16*, 27–32.

Yamamoto, M., Yaginuma, K., Tsutsui, H., Sagara, J., Guan, X., Seki, E., Yasuda, K., Yamamoto, M., Akira, S., Nakanishi, K., et al. (2004). ASC is essential for LPS-induced activation of procaspase-1 independently of TLR-associated signal adaptor molecules. Genes Cells *9*, 1055–1067.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., and Fujita, T. (2004). The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. *5*, 730–737.

Zhao, Q., Wang, X., Nelin, L.D., Yao, Y., Matta, R., Manson, M.E., Baliga, R.S., Meng, X., Smith, C.V., Bauer, J.A., et al. (2006). MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. J. Exp. Med. *203*, 131–140.