

Review

Bacterial interference with canonical NF κ B signalling

Mona Johannessen, Fatemeh Askarian, Maria Sangvik and Johanna E. Sollid

Correspondence

Mona Johannessen
mona.johannessen@uit.no

Research Group of Host–Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

The human body is constantly challenged by a variety of commensal and pathogenic micro-organisms that trigger the immune system. Central in the first line of defence is the pattern-recognition receptor (PRR)-induced stimulation of the NF κ B pathway, leading to NF κ B activation. The subsequent production of pro-inflammatory cytokines and/or antimicrobial peptides results in recruitment of professional phagocytes and bacterial clearance. To overcome this, bacteria have developed mechanisms for targeted interference in every single step in the PRR–NF κ B pathway to dampen host inflammatory responses. This review aims to briefly overview the PRR–NF κ B pathway in relation to the immune response and give examples of the diverse bacterial evasion mechanisms including changes in the bacterial surface, decoy production and injection of effector molecules. Targeted regulation of inflammatory responses is needed and bacterial molecules developed for immune evasion could provide future anti-inflammatory agents.

Introduction

Micro-organisms colonize all available surfaces of the human body. The immune sentinels of the skin and mucosa recognize both commensals and pathogens with the intention to dampen colonization and potential invasion. Highly conserved bacterial structures, termed pathogen associated molecular patterns (PAMPs) are recognized by sensors such as pattern-recognition receptors (PRRs). Among the PRRs are the Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), RIG-I-like receptors (RLRs) and C-type lectin receptors (CLRs). A microbe can be

recognized by independent PRRs simultaneously, and the sensors activate various signal cascades that generally converge to transcription factors including NF κ B, activator protein (AP)-1, nuclear factor of activated T cells (NFAT) and interferon regulatory factors (IRFs), which then activates transcription of immunity-related genes (Nish & Medzhitov, 2011; Lee & Kim, 2007).

Bacteria have evolved various immune evasive mechanisms such as prevention of complement deposition, blockade of antibody function and dampening of neutrophil recruitment by production of decoy molecules that disturb the chemotactic gradient (Bestebroer *et al.*, 2010; Veldkamp & van Strijp, 2009). Central in the first line of defence against bacteria is the recognition of the bacteria by the PAMP–PRR. However, different bacteria, both pathogenic and non-pathogenic, vary in the ability to induce production of immunity-related molecules (Wanke *et al.*, 2011; Neish *et al.*, 2000). This may be explained by species- or strain-dependent variation in ability to interfere with early host cell signalling. Such an early inhibitory mechanism may be of importance for survival as a commensal or in promoting bacterial infection due to decline in the first induced pro-inflammatory responses (Ashida *et al.*, 2010). In this review, we aim to give some examples of how bacteria can modulate their recognition by interfering with canonical host cell signalling to NF κ B.

Canonical NF κ B signalling and the immune response

NF κ B proteins are a family of five structurally related transcription factors: RelA/p65, RelB, c-Rel and the

Abbreviations: CXCR4, CXC-chemokine receptor 4; DD, death domain; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-ligating enzyme; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; IL-1R, interleukin-1 receptor; I κ B, inhibitor of κ B; ITIM, immune-receptor tyrosine-based inhibition motif; MyD88, myeloid differentiation factor 88; MAL, MyD88-adaptor like; NEMO, NF κ B essential modifier; NES, nuclear export signal; NLS, nuclear localization signal; NOD, nucleotide oligomerization domain; NLR, NOD-like receptor; PAMP, pathogen-associated molecular pattern; PRR, pattern-recognition receptor; PPAR- γ , peroxisome proliferator activated receptor- γ ; Opa, opacity-associated protein; RHD, Rel homology domain; RPS3, ribosomal protein S3; SARM, sterile α - and armadillo-motif-containing protein; SseL, *Salmonella typhimurium* secreted factor L; SSL, staphylococcal superantigen-like protein; TAB, TAK1 binding protein; TAD, transactivation domain; TAK1, growth factor- β -associated kinase-1; TCR, T-cell receptor; TIR, Toll/interleukin-1 receptor; Tir, intimin receptor; TLR, Toll-like receptor; TNFR, tumour necrosis factor receptor; TRAF, tumour necrosis factor receptor-associated factor; TRIF, TIR-domain-containing adaptor protein including interferon- β ; TRAM, TRIF-related adaptor molecule; UspA1, ubiquitous surface protein A1; UTI, urinary tract infection; YopJ, *Yersinia* outer protein J.

proteins synthesized as precursors, p50 (p105/NFκB1) and p52 (p100/NFκB2). They all contain a conserved Rel homology domain (RHD) that contains a nuclear localization signal (NLS), directing the transcription factors into the nucleus. Moreover, the RHD is involved in the homo- or heterodimerization of the transcription factors, and in their binding to DNA at κB sites that are localized within target gene promoters (Li & Verma, 2002).

NFκB is sequestered in the cytosol by inhibitor of κB (IκB) in resting cells, and appropriate stimuli are needed to induce its translocation to the nucleus. The canonical signalling pathway to NFκB is induced by stimuli that activate the tumour necrosis factor receptor (TNFR), the interleukin-1 receptor (IL-1R), the T-cell receptor (TCR), TLRs and the NLRs NOD1 and NOD2 (Oeckinghaus *et al.*, 2011; Sun, 2012). All the aforementioned receptors use various adaptors and signalling molecules, but the signalling converges at the IκB kinase (IKK) complex containing IKKα, IKKβ and NFκB essential modifier (NEMO), upstream of IκB and NFκB (Oeckinghaus *et al.*, 2011; Sun, 2012) (Fig. 1).

Ten members of the TLR protein family are found in humans and include the cell membrane-bound TLR1, TLR2, TLR4, TLR6 and the endosome-associated TLR3,

TLR7, TLR8 and TLR9 (Casanova *et al.*, 2011; Nestle *et al.*, 2009). TLRs belong to the Toll-like/IL-1 receptor superfamily that shares an intracellular domain referred to as the Toll/interleukin-1 receptor (TIR) domain. Engagement of TLRs by PAMPs activates an intracellular cascade resulting in induction of pro-inflammatory genes (Fig. 2). More specifically, the PAMP-TLR interaction results in receptor-dimerization via the TIR domain, and/or a conformational change. The TIR domain of the receptors then attracts TIR-containing adaptor proteins such as myeloid differentiation factor 88 (MyD88), MyD88-adaptor like (MAL, also called TIRAP), TIR-domain-containing adaptor protein including interferon-β (TRIF), TRIF-related adaptor molecule (TRAM) or sterile α- and armadillo-motif-containing protein (SARM). TLR recruitment of the adaptors, except SARM, activates the signal cascade that subsequently recruits downstream signalling molecules (Watters *et al.*, 2007). MyD88 either binds TLRs directly (TLR5, TLR7, TLR8, TLR9) or indirectly (TLR1, TLR2, TLR4, TLR6) through MAL/TIRAP (O'Neill & Bowie, 2007). MyD88 comprises an N-terminal death domain (DD) and a C-terminal TIR-domain, separated by a small intermediate domain (ID) (Watters *et al.*, 2007). MyD88 interacts with TLRs via the TIR domain, while the DD of MyD88 associates with the DD of the IL-1R-associated kinases

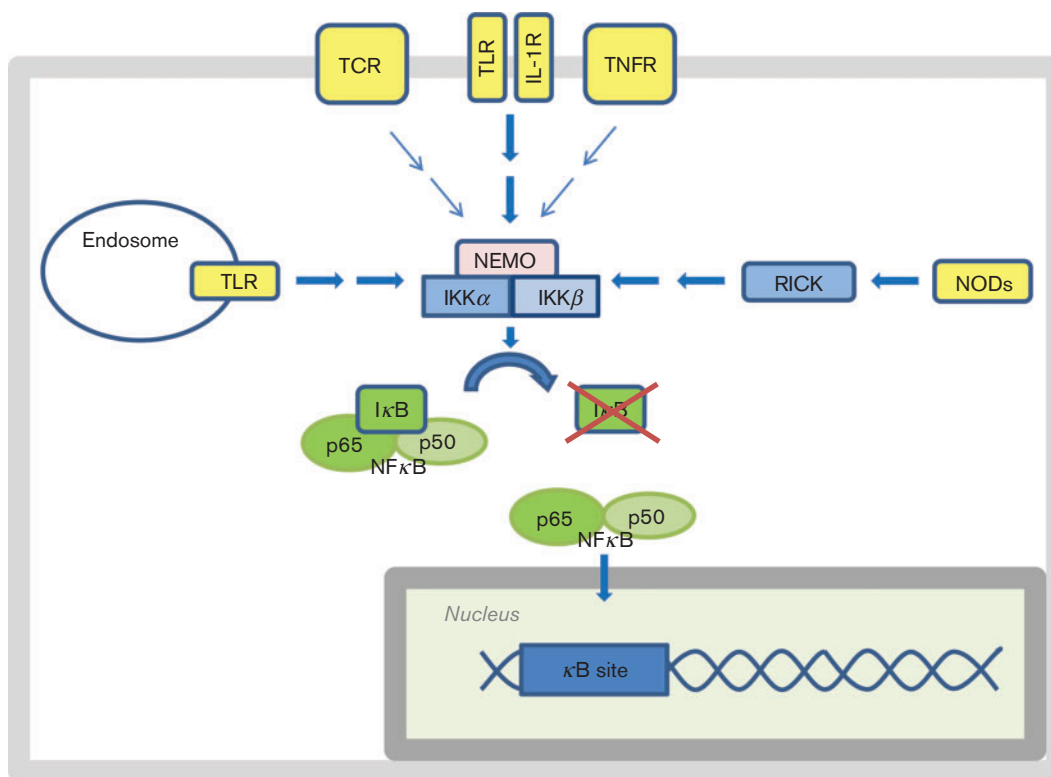


Fig. 1. Activation of the canonical signalling pathway to NFκB results in nuclear translocation of the transcription factor. NFκB is sequestered in the cytosol in resting cells due to binding to IκB. IκB can be degraded (illustrated by the red cross) upon signalling from the activated NEMO/IKKα/IKKβ complex (IKK complex). The IKK complex can be activated by various stimuli that engage the TCR, TLRs, IL-1R, TNFR and NLRs.

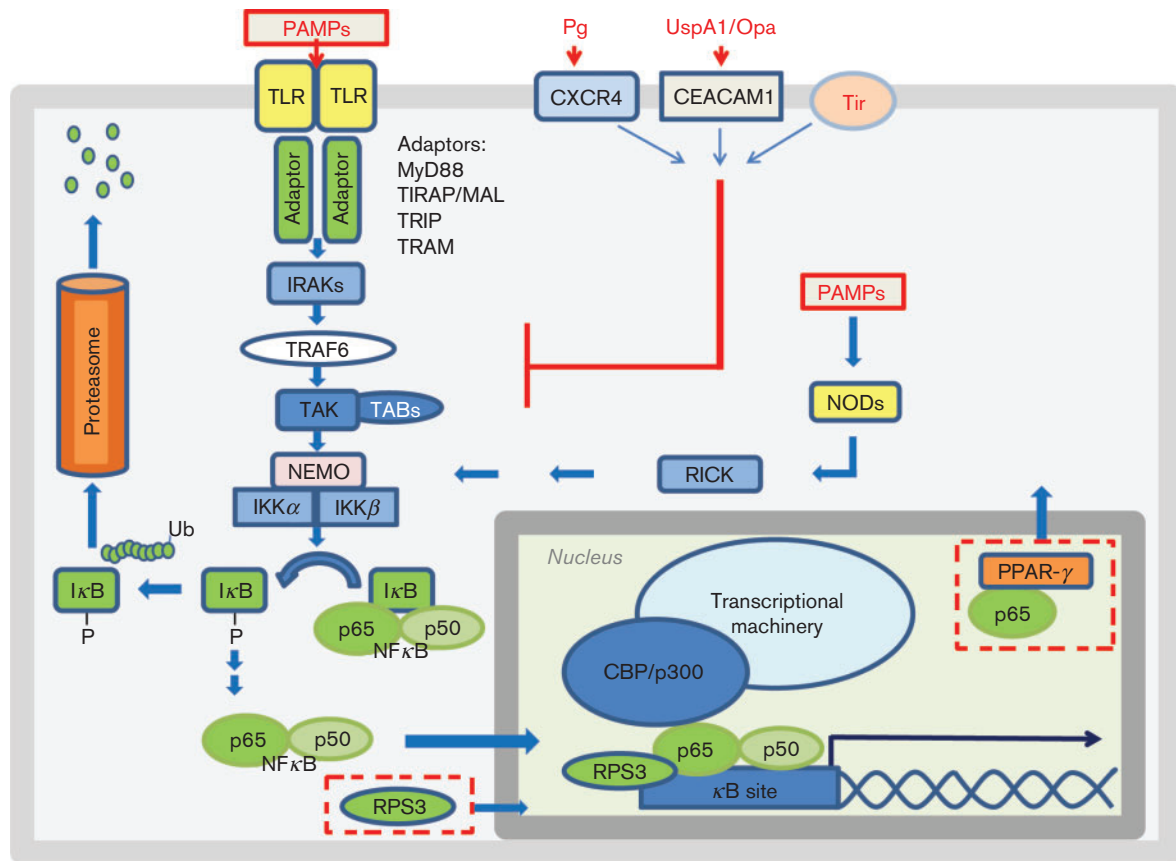


Fig. 2. Engagement of TLRs or NODs by PAMPs results in NFκB activation. PAMPs induce conformational changes in TLRs, resulting in recruitment of appropriate adaptors for example MyD88/MAL. The adaptors recruit the IRAKs which autophosphorylate thereby attracting TRAF6 which again activates the TAK complex resulting in stimulation of the NEMO/IKKα/IKKβ complex (IKK complex). The activated IKK complex phosphorylates IκB, targeting it for ubiquitination and proteasomal degradation. NFκB can then enter the nucleus where it binds to the κB site on appropriate promoters, and the binding can be improved by RPS3. The TAD of p65 recruits co-activators such as CBP/p300 which results in establishment of the transcriptional machinery and transcription of genes encoding proteins involved in immunity. The bacterial surface structure proteins Pg-fimbriae and UspA1/Opa stimulate CXCR4 and CEACAM1 receptors thereby activating cascades that negatively interfere with TLR-induced NFκB activation. Injected *E. coli* Tir protein inhibits the cascade with a similar mechanism. Nuclear proteins can also be targeted by bacterial effectors. Bacterial effectors can prevent translocation of RPS3 into the nucleus. Similarly, bacterial presence can induce PPAR-γ-mediated nuclear translocation of p65. In the figure, bacterially derived molecules are written in red. The bacterially induced negative interference in signalling pathways or localization of cellular proteins is illustrated with red lines or boxes with red dashed lines, respectively. Ub, ubiquitin; P, phosphate; CBP/p300, CREB binding protein/p300; TAD, transactivation domain.

IRAK1, IRAK2 and IRAK4. The kinases are then activated due to auto- and cross-phosphorylation. This leads to recruitment of tumour necrosis factor receptor-associated factor (TRAF)6, followed by a complex consisting of growth factor-β-associated kinase-1 (TAK1), TAK1 binding protein (TAB)1 and TAB2. TAK1 can phosphorylate IKKβ, leading to activation of the IKK complex. Next, the IKK complex phosphorylates IκB, which then is recognized by a subunit of the ubiquitin E3 ligase complex SCF^{βTrCP}. This leads to K48-polyubiquitination of IκBα, which targets it for degradation by the proteasome (Jiang & Chen, 2012; Watters *et al.*, 2007; Chen, 2005). When IκBα is degraded, the NLS of NFκB subsequently becomes

available, and the transcription factor can translocate to the nucleus. In the nucleus NFκB can bind to κB sites, thereby influencing gene expression with impact on immune responses (Sun, 2012; Newton & Dixit, 2012; Kumar *et al.*, 2009) (Fig. 2).

Activation of TLRs and other PRRs are associated with the risk of chronic inflammatory diseases and immune disorders, and the pathway needs to be highly regulated. Endogenous inhibition of TLR signalling can occur at various levels. The level of TLRs can be reduced either by increased degradation or by reduced production, or the receptor-induced signalling can be inhibited by e.g. the

host encoded orphan receptor ST2 (Jeong & Lee, 2011; Han & Ulevitch, 2005). Inhibition of the pathway can also be induced. For example, during chronic conditions of inflammation or prolonged exposure to LPS, a splice variant of MyD88 named MyD88s is produced. MyD88s acts as a negative regulator of MyD88 function and blocks its signalling (Burns *et al.*, 2003; Janssens *et al.*, 2002). Similarly, IRAK-M is another example of an endogenously produced antagonist of TLR pathways that inhibits signalling at the level of IRAKs (Han & Ulevitch, 2005).

The importance of TLR signalling in immune defence can also be observed by studying naturally occurring genetic mutations or polymorphisms in innate immune genes among humans. Since 2003, 24 patients with MyD88 deficiencies and 52 patients with IRAK4 deficiencies have been followed. The deficiencies are life-threatening during childhood, and the paediatric patients have a predisposition to severe bacterial infection often caused by pyogenic Gram-positive bacteria, such as *Streptococcus pneumoniae* and *Staphylococcus aureus* (von Bernuth *et al.*, 2012). Similar effects are seen in patients with mutations that abrogate the genes encoding NEMO and $\text{I}\kappa\text{B}\alpha$ (Suhir & Etzioni, 2010; Turvey & Hawn, 2006). Also, certain polymorphisms in TLRs or signalling components affect susceptibility to infection. Humans with TLR2 Arg753Gln show increased susceptibility to tuberculosis and infections by Gram-positive organisms. Similarly, individuals with TLR4 Asp299Gly or Thr399Ile are hypo-responsive to LPS (Carpenter & O'Neill, 2007).

These studies demonstrate that PRR- and MyD88-induced signalling is of importance in protection against various bacteria especially during childhood. Therefore, the presence of immune evasion mechanisms targeting this signalling pathway is not surprising. This strategy may allow the bacteria to survive as commensals (Xavier & Podolsky, 2000), or to stay un-recognized for as long as possible during infection.

Bacterial evasion mechanisms targeting PRR–NF κ B signalling

Evasion of recognition by pattern recognition receptors.

One can imagine several mechanisms which could interfere with TLR signalling. One approach is to avoid the recognition of PAMPs, exemplified by a mechanism involving a polysaccharide capsule on the outermost surface of the bacteria. In the case of *Enterococcus faecalis*, the complement factor C3 is deposited on the surface of capsular serotypes C and D, as well as on the non-encapsulated strain. However, the presence of the capsule masked the complement from detection by anti-C3 antibodies and protected against opsonophagocytosis. Similarly, the PAMPs LTA and peptidoglycan were masked from detection by antibodies in encapsulated strains, and these strains showed reduced TNF- α production by the macrophage cell line RAW264.7 (Thurlow *et al.*, 2009). A similar mechanism is used by *Staph. aureus* (Nanra *et al.*, 2012; O'Riordan & Lee, 2004). The efficiency of

masking the bacterial cell surface may be dependent on the particular capsule type expressed, as for example the capsule of *Strep. pneumoniae* type 3 strain WU2 masked surface antigens more effectively than the type 2 D39 strain (Abeyta *et al.*, 2003).

A second approach is to modify the surface of PAMPs, thereby interfering with their recognition by pattern recognition receptors such as TLRs or NODs. This strategy is used, for example, by *Salmonella typhimurium*. Presence of the host is recognized by the bacterial sensor histidine kinase PhoP localized on the bacterial surface, which then phosphorylates the intracellular effector molecule PhoQ which results in bacterial expression of lipid A modifying enzymes. The modified version of lipid A has reduced ability to induce TLR4-mediated NF κ B-activation (Kawasaki *et al.*, 2004). *Pseudomonas aeruginosa* also modifies its lipid A and muropeptide during chronic cystic fibrosis infection, resulting in reduced cytokine secretion and leukocyte recruitment (Cigana *et al.*, 2009). Similar strategies are used by *Francisella tularensis* and *Mycobacterium tuberculosis*. The lipid A of the former is hypo-acetylated (tetra-acetylated) and not properly recognized by TLR4 (Gunn & Ernst, 2007), while the cell envelope-associated serine hydrolase Hip1 of *My. tuberculosis* restricts the onset and magnitude of pro-inflammatory cytokines probably by limiting recognition of TLR2 ligands (Madan-Lala *et al.*, 2011). When the bacteria are inside the host cell, peptidoglycan breakdown products become available for detection by the PRRs NOD1 and NOD2. NOD engagement of PAMPs activates receptor interacting serine/threonine kinase (RICK), resulting in activation of NF κ B-mediated transcription (Kersse *et al.*, 2011) (Fig. 2). Several bacteria modify peptidoglycan in order to avoid being recognized by NODs (Wolfert *et al.*, 2007), and *Legionella pneumophila* encodes a periplasmic protein EnhC that inhibits degradation of peptidoglycan thereby preventing production of PAMPs (Liu *et al.*, 2012).

A third approach is to inhibit TLR signalling by direct interactions between secreted bacterial effector molecules and the host TLR receptors. *Staph. aureus* may express and secrete staphylococcal superantigen-like proteins (SSLs) and several of the SSLs have immune modulating properties (Fraser & Proft, 2008). Recently SSL3, and to a certain extent SSL4, were found to negatively interfere with TLR2-mediated production of IL-8 (Bardoel *et al.*, 2012). The inhibitory effect is most likely due to competition between SSL3 and the PAMP ligands of TLR2. The inhibition is partly dependent on sialic residues on TLR2 (Bardoel *et al.*, 2012; Yokoyama *et al.*, 2012).

A fourth approach is to activate a counteracting signalling pathway which negatively interferes with the TLR-signalling cascade (Fig. 2). *Porphyromonas gingivalis* has surface bound fimbriae often referred to as Pg-fimbriae. Pg-fimbriae can interact with TLR2/CD14 resulting in increased NF κ B activity. However, Pg-fimbriae simultaneously interact with an additional host receptor, CXC-chemokine receptor 4 (CXCR4), resulting in intracellular

cross-talk between two different signalling pathways (e.g. the cAMP/PKA pathway and the TLR2 pathway) which leads to impaired NF κ B activation and host defence (Hajishengallis *et al.*, 2008). *Moraxella catarrhalis* and *Neisseria meningitidis* use a similar strategy with their ubiquitous surface protein A1 (UspA1) and opacity-associated protein (Opa), respectively. These structurally unrelated proteins can both interact with the Ig-like CEACAM1 receptor of host cells (Slevogt *et al.*, 2008). The intracellular part of CEACAM1 composes an immune-receptor tyrosine-based inhibition motif (ITIM). When ITIM-bearing receptors engage their ligands, intracellular tyrosine residues becomes phosphorylated resulting in recruitment of phosphotyrosine phosphatases such as SHP-1, SHP-2 or the inositol phosphatase SHIP. The TLR adaptors TIRAP and MyD88 can be tyrosine phosphorylated, suggesting that ITIM can have a regulatory role in TLR signalling (Barrow & Trowsdale, 2006). Indeed, the interaction between bacterial UspA1/Opa and host CEACAM1 results in recruitment of an intracellular phosphatase which negatively regulates TLR2-dependent activation of the NF κ B pathway (Slevogt *et al.*, 2008). Similarly, *Staph. aureus* is found to interact with paired Ig-like receptors containing ITIMs on mouse macrophages and thereby negatively interfere with TLR-mediated cytokine production in these cells (Nakayama *et al.*, 2007). In either case, the net result of the interaction with ITIM-containing receptors is that activation of NF κ B is inhibited. The above-mentioned examples include bacterial cell surface proteins that interact with an ITIM-containing host receptor. In contrast, enteropathogenic *Escherichia coli* (EPEC) uses a strategy where it injects bacterial intimin receptor (Tir) into the epithelial cell membrane. The extracellular part of Tir is engaged by the bacterial surface ligand intimin, while the intracellular part of Tir contains a region with similarity to host ITIMs (Yan *et al.*, 2012). The ITIMs of the bacterial Tir protein interact with the eukaryotic tyrosine phosphatase SHP-1, and enhance its binding to TRAF6. The ubiquitination and activation of TRAF6 is inhibited, and EPEC-induced expression of pro-inflammatory cytokines is suppressed (Yan *et al.*, 2012) (Fig. 2).

How to prevent recruitment and activity of the adaptor molecules? Activated Toll-like receptors recruit the appropriate intracellular adaptors MyD88, MAL/TIRAP, TRIF or TRAM (Newton & Dixit, 2012; Takeda & Akira, 2004) (Fig. 2). One mechanism to evade signal transduction at this stage is to interfere with the stability of the relevant adaptor proteins. *Brucella* spp. encode a protein named TcpB/Btp1 that negatively interferes with NF κ B-activity *in vitro* (Cirl *et al.*, 2008; Radhakrishnan *et al.*, 2009; Salcedo *et al.*, 2008; Sengupta *et al.*, 2010). TcpB mimics the membrane binding properties of TIRAP/Mal and localizes to the cellular plasma membrane (Radhakrishnan *et al.*, 2009). TcpB can also interact with the adaptor MAL/TIRAP, thereby inducing its ubiquitination and degradation (Sengupta *et al.*, 2010), and this prevents host signal transduction.

All the TLRs, IL-1R and the adaptors MyD88, TIRAP/Mal, TRIF, TRAM and SARM share an intracellular conserved region called the TIR domain (Watters *et al.*, 2007). The TIR domain is approximately 200 amino acids in length and sequence conservation is generally in the 20–30% range (Xu *et al.*, 2000). Crystallographic analysis of the TIR domains of human TLR1, TLR2 and TLR10 has shown that the TIR-domain is composed of five β -sheets (β A– β E) surrounded by five α -helices (α A– α E). The loop connecting β B and α B, named the BB-loop, forms a protrusion that extends from the surface of the TIR domain. Three conserved amino acid motifs named boxes 1, 2 and 3 exist, and the BB-loop comprises a part of box 2, which is of importance in eukaryotic signal transduction (Li *et al.*, 2005; Xu *et al.*, 2000; Watters *et al.*, 2007). Indeed, replacement of proline 712 in TLR4 or proline 681 in TLR2 with histidine results in receptors that do not respond to LPS or Gram-positive bacteria (Hoshino *et al.*, 1999; Poltorak *et al.*, 1998; Underhill *et al.*, 1999). In the case of TLR2, studies have shown that the mutation P681H prevents its interaction with MyD88, thereby blunting further signal transduction (Xu *et al.*, 2000). The TIR–TIR interaction is therefore of importance in signalling, and another immune evasion strategy is to interfere with this. Indeed, Cirl and coworkers found that the uropathogenic *E. coli* CTF073 encodes a TIR-containing protein named TcpC, which via its binding to MyD88 negatively interferes with TLR4–MyD88–NF κ B signalling (Cirl *et al.*, 2008; Yadav *et al.*, 2010). Urinary tract infection (UTI) studies in mice revealed that *E. coli* with TcpC could multiply to higher numbers than TcpC-deficient strains, and molecular epidemiological studies among human UTI patients revealed that TcpC was associated with severity of disease (Cirl *et al.*, 2008). An intact BB loop of the bacterial TIR domain is of importance for its function, e.g. the proline residue in the exposed BB loop of *Yersinia pestis* YpTIR was needed for the interaction with MyD88, as the Pro173His mutant of YpTIR did not interfere with LPS-induced signalling (Spear *et al.*, 2012). Similarly, mutation of a particular glycine in the BB-loop of *Brucella* TcpB resulted in reduced ability to suppress NF κ B (Radhakrishnan *et al.*, 2009). Furthermore, database searches have shown that TIR domains are found in hundreds of bacterial species (Spear *et al.*, 2009; Zhang *et al.*, 2011a), and phylogenetic analysis reveals that SARM, which is the only TIR-containing adaptor that negatively regulates TLR signalling, is clustered together with bacterial TIR proteins (Zhang *et al.*, 2011a).

Can bacteria interfere with IRAKs, TRAFs or TAK/TAB complexes? The TLR/MyD88 interaction makes the DD of MyD88 available for interaction with the DD of serine/threonine kinase IRAK4, which via its DD domain binds to the DD domain of the related kinases IRAK1 and IRAK2. MyD88 and IRAKs nucleate a larger complex, which in the case of TLR4 signalling includes TRAF6 (Newton & Dixit, 2012) (Fig. 2).

IRAK-M is an endogenous inhibitor of IRAK activity (Han & Ulevitch, 2005) and IRAK-M-deficient mice had reduced lethality after infection with *Strep. pneumoniae* (van der Windt *et al.*, 2012). Reduced lethality in the absence of the negative regulator IRAK-M reveals that inhibition of the pathway is beneficial for the microbe. One approach for a microbe to hijack signalling could be to induce expression of endogenous inhibitors in host cells. The mammalian intestinal tract harbours a large number of commensal bacteria which could also be recognized via their PAMPs. However, commensals were found to induce expression of IRAK-M in the intestine of BALB/c mice, and the authors suggest that the induction of a negative regulator of TLR signalling plays a role in homeostasis of the intestine and survival of the microbes (Biswas *et al.*, 2011). Induced IRAK-M expression is also found in THP-1-derived macrophages after exposure to LPS from *Po. gingivalis*. The resultant reduced production of the pro-inflammatory cytokines TNF α , IL-6 and IL-12p40 may be advantageous for the bacteria, causing chronic infection during periodontitis (Domon *et al.*, 2008).

The next level in the signalling pathway involves TRAF6 and TAB/TAK (Fig. 2). The function of the E3 ubiquitin ligase TRAF6 can also be a target for bacterial immune evasion. Ubiquitin is a conserved 76 amino acid peptide in eukaryotes that can be covalently attached to other proteins through an enzymatic process involving three sequential steps performed by one ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2s) and ubiquitin-ligating enzymes (E3s). Host proteins can be modified by addition of polyubiquitin chains, and K63-linked polyubiquitination is often involved in signal transduction. When induced, for instance due to the presence of a PAMP (Fig. 2), the signalling cascade results in activation of TRAF6 that performs K63-polyubiquitination of itself. This leads to recruitment of zinc-finger ubiquitin-binding proteins TAB2 and TAB3, which are regulators of the TAK1 complex (Jiang & Chen, 2012). One option for evasion of the cascade is to prevent polyubiquitination in the first place. The E3 ubiquitin ligase function of TRAF6 is dependent on E2 ubiquitin-conjugating enzymes such as UBC13. This particular E2 conjugating enzyme is a target for *Shigella flexneri* OspI, which deamidates the UBC13 glutamine residue at position 100 into glutamate. Consequently, the E2 conjugating activity which is required for TRAF6 activation is inhibited (Sanada *et al.*, 2012). Another option for evasion is to remove ubiquitin-chains that were already placed on target proteins. Indeed, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* encode a de-ubiquitinating protease named *Yersinia* outer protein J (YopJ). Bacterial YopJ in host cells results in removal of K63-polyubiquitination of TRAF6. As the K63-polyubiquitination of TRAF6 is a signal for recruitment of the NEMO/IKK α /IKK β complex and the TAK1/TAB1/TAB2 complex, the YopJ induced de-ubiquitination blocks the host intracellular signalling to NF κ B (Zhou *et al.*, 2005; Sweet *et al.*, 2007).

Recruitment of the TAB/TAK complex to the upstream protein TRAF6 (Fig. 2) depends on the ability of TAB to

recognize and bind to the K63-polyubiquitination that appears after stimulation by PAMPs. Bacteria have also evolved a mechanism to interfere with the binding properties of TABs, thereby preventing the recruitment and activation of the TAB/TAK complex. For example, EPEC-encoded NleE was found to harbour a S-adenosyl-L-methionone-dependent methyltransferase activity. NleE specifically methylates the zinc-coordinating cysteine in the Npl4 zinc finger domains of TAB2 and TAB3. The methylation of the cysteine residue of the TABs inhibits their ability to bind to the ubiquitin chain, and the signalling pathway is dampened (Zhang *et al.*, 2011b).

Can bacterial effectors interfere with IKK complexes?

Downstream of the IRAKs and TRAF6 are the TAK/TAB complexes that, when appropriate, activate IKK complexes (Fig. 2). *Yersinia* YopJ also has acetyltransferase activity, and the protein can acetylate IKK α and IKK β on a threonine residue within their activation loop, thereby preventing their modification and activation by phosphorylation. This results in an inactive IKK complex which then dampens the signal to NF κ B even in presence of PAMPs (Mittal *et al.*, 2006).

The IKK complex is also targeted by other bacteria. *Shigella* encode a bacterial E3 ubiquitin ligase IpaH9.8 that uses the A20-binding inhibitor of NF κ B (ABIN-1) as an adaptor in order to K27-polyubiquitinate NEMO/IKK γ . The bacterial E3-induced polyubiquitination of NEMO/IKK γ results in degradation of the complex, and activation of NF κ B is then disturbed. Mice infected intranasally with wild-type but not IpaH9.8-deficient *Shigella* show earlier reduction of inflammation in the lung. Consistent with this, MIP-2, IL-6, IL-1 β and tissue myeloperoxidase level were higher in mice infected with IpaH9.8-deficient strains. IpaH9.8 was especially functional in inhibiting NOD1-induced NF κ B activation, but could also inhibit the TLR4 pathway (Ashida *et al.*, 2010). In either case, the early induction of pro-inflammatory cytokines could be inhibited in the presence of the bacterial E3 ligase IpaH9.8.

Do bacteria interfere with the ability of I κ B α to retain NF κ B in the cytoplasm?

The activation of TLR signalling results in phosphorylation followed by K48-polyubiquitination of I κ B α , which targets I κ B α for proteasomal destruction. The released NF κ B complex can then enter the nucleus (Jiang & Chen, 2012) (Fig. 2).

One mechanism to blunt signalling at this stage is to negatively interfere with the ubiquitination process directly, that is by interfering with E1, E2 or E3 enzymes. The *Shig. flexneri* effector OspG targets the E2 ubiquitin-conjugating enzyme UbcH5, which is used by the E3 ligase complex SCF ^{β T α CP}. The result of the OspG-UbcH5 interaction is inhibition of polyubiquitination and subsequent degradation of I κ B, resulting in suppression of the signalling pathway. The ligated ileal loop model of infection in rabbits was used to compare infection with wild-type versus an *ospG* mutant. The mutant induced stronger destruction of the mucosa than the wild-type *Shigella*, i.e. more necrotic tissue

was found and the villi were almost completely eliminated. Hence the lack of *ospG* resulted in a stronger inflammatory response upon infection *in vivo* (Kim *et al.*, 2005). This clearly illustrates the effectiveness of OspG in evading induction of pro-inflammatory genes.

Another mechanism to interfere with I κ B's ability to retain NF κ B in the cytoplasm is to remove the K48-ubiquitin labelling from I κ B that otherwise targets it for proteasomal degradation. This mechanism is used by *Yersinia* using the deubiquitinating protease YopJ (Zhou *et al.*, 2005). The net result is that I κ B remains intact and retains the ability to sequester NF κ B in the cytoplasm, thereby preventing activation of NF κ B responsive genes. *Sal. typhimurium* secreted factor L (SseL) has a similar function, and comparison of infection outcomes in mice showed that the presence of SseL reduced the *in vivo* inflammatory response. For example, four days post-infection the livers of mice infected with Δ *sseL* had fourfold more granulomas than livers from mice infected with wild-type bacteria. Similarly, the lumen, submucosa and surface epithelium had greater PMN (polymorphonuclear neutrophil) infiltration in mice infected with *Sal. typhimurium* Δ *sseL* compared to wild-type strain. The mice infected with the mutant strain died earlier than those infected with the wild-type strain (Le Negrate *et al.*, 2008).

Various *Salmonella* strains differ in their ability to interfere with IL-8 secretion. *Sal. typhimurium* induced IL-8 secretion in epithelial cells, while the non-pathogenic laboratory-derived *Sal. typhimurium* PhoP^c and naturally occurring *Salmonella pullorum* did not. The non-pathogenic strains induced phosphorylation of I κ B but no ubiquitination of I κ B was achieved and the pathway was blunted (Neish *et al.*, 2000). The bacterial effector is AvrA, which acts as a deubiquitinase and reduces inflammatory responses as well as host cell apoptosis (Ye *et al.*, 2007; Collier-Hyams *et al.*, 2002).

In contrast to the above examples where the bacterial effector negatively interferes with the signal cascade, the *Legionella pneumophila* effectors LnaB and LegK1 do the opposite (Ge *et al.*, 2009; Losick *et al.*, 2010). *L. pneumophila* is a facultative intracellular pathogen that infects human alveolar macrophages and epithelial cells in the respiratory tract, leading to a severe pneumonia known as Legionnaires' disease. Activation of the pathway has been suggested to positively contribute to intracellular growth by inducing genes encoding antagonists of apoptosis (Losick *et al.*, 2010). LegK1 mimics eukaryotic serine/threonine kinases and can directly phosphorylate I κ B on serine-52 and serine-36, resulting in activation of an NF κ B reporter (Ge *et al.*, 2009).

Bacterial effectors may interfere with the function of NF κ B

In the nucleus, the transcription factor NF κ B binds to appropriate κ B sites located in available promoters of

various genes encoding proteins involved in immune defence (Newton & Dixit, 2012) (Fig. 2). The availability of κ B sites for transcription factors can depend on various parameters such as condensation of the DNA. Eukaryotic DNA is wrapped around four core histones (H2A, H2B, H3 and H4). The histones have an N-terminal part that protrudes out of the nucleosome, and is available for post-translational modifications. These modifications determine whether the DNA is available for transcription, i.e. phosphorylation of histone 3 (H3) increases the availability of κ B sites in the promoter of IL-8. OspF from *Shig. flexneri* is a dual specific phosphatase that localizes to the nucleus where it dephosphorylates and thereby inactivates the MAPK kinases involved in H3 phosphorylation. This results in reduced H3 phosphorylation in the *IL-8* gene resulting in reduced binding of NF κ B and the RNA polymerase II complex to the DNA. OspF also restricts bacterial invasion and prevents PMN recruitment in a ligated ileal loop rabbit model of infection (Arbibe *et al.*, 2007).

RelA/p65, RelB and c-Rel have a transactivation domain (TAD) involved in transactivation of target genes. In contrast, p50 and p52 can bind κ B sites but lack the TAD, resulting in transcriptional repression (Li & Verma, 2002). Therefore, another strategy to modulate transcription is to interfere with which type of subunit of NF κ B actually enters the nucleus. EPEC and enterohaemorrhagic *E. coli* (EHEC) encoding NleE were found to inhibit nuclear translocation of transactivating subunits p65 and c-Rel, but not the repressor p50, leading to reduced IL-8 production (Newton *et al.*, 2010). This suggests that one putative mechanism to disturb the activity of NF κ B is to interfere with which subunits enter the nucleus (Fig. 2). A similar strategy has been used by *Bordetella pertussis*. These bacteria encode BopN that promotes the translocation of the repressive p50 subunit. The translocation of the repressive subunit resulted in increased expression of the anti-inflammatory IL-10, which may be an advantage for persistent colonization (Nagamatsu *et al.*, 2009). Another mechanism is to degrade subunits containing TADs. EPEC- and EHEC-encoded NleC is a zinc-dependent protease that degrades NF κ B protein p65, resulting in reduced IL-8 secretion (Yen *et al.*, 2010; Pearson *et al.*, 2011; Baruch *et al.*, 2011; Mühlen *et al.*, 2011).

Appropriate localization of NF κ B is needed for its function. I κ B contains a nuclear export signal (NES), and shuttles between the nucleus and cytoplasm. Binding of I κ B to nuclear NF κ B hides the NLS signal of the transcription factor and the NES signal is exposed. This leads to nuclear export receptor exportin-1-mediated transport of the I κ B–NF κ B complex from the nucleus to the cytoplasm (Huang *et al.*, 2000). One anti-inflammatory strategy used by bacteria is to target the localization of NF κ B subunits. *Bacteroides thetaiotaomicron* is a prevalent anaerobe of the human intestine which can attenuate inflammation caused by the pathogenic *Salmonella enterica* serovar Enteritidis. Both bacteria induce expression of peroxisome proliferator

Table 1. Immune evasion mechanisms at various steps in the TLR–NF κ B pathway

| Targeted step in the pathway | Bacterial species | Bacterial molecule/effector | Mechanism of action | Reference |
|------------------------------|-------------------------------------------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
| TLRs | <i>En. faecalis</i> | Capsule | Masks PAMPs from detection | Thurlow <i>et al.</i> , 2009 |
| | <i>Staph. aureus</i> | Capsule | Masks PAMPs from detection | Nanra <i>et al.</i> , 2012; O’Riordan & Lee, 2004 |
| | <i>Strep. pneumoniae</i> | Capsule | Masks PAMPs from detection | Abeyta <i>et al.</i> , 2003 |
| | <i>Ps. aeruginosa</i> | Lipid A | <i>pagL</i> mutation and modification of lipid A over prolonged infection | Cigana <i>et al.</i> , 2009 |
| | <i>Sal. typhimurium</i> | Lipid A | Induced expression of lipid A modifying enzymes | Kawasaki <i>et al.</i> , 2004 |
| | <i>My. tuberculosis</i> | Hip1 | Limits the recognition of TLR2 ligands | Madan-Lala <i>et al.</i> , 2011 |
| | <i>Staph. aureus</i> | SSL3 | Binds to and inhibits TLR2 function | Bardoel <i>et al.</i> , 2012; Yokoyama <i>et al.</i> , 2012 |
| | <i>Po. gingivalis</i> | Pg | Binds CXCR4, thereby counteracting TLR2 effect | Hajishengallis <i>et al.</i> , 2008 |
| | <i>Mo. catarrhalis</i> , <i>N. meningitidis</i> | UspA1, Opa | Binds CEACAM1 and recruits SHP-1, which negatively interferes with signalling (dephosphorylates the p85 α binding motif of TLR2) | Slevogt <i>et al.</i> , 2008 |
| TIRAP/MyD88 | <i>E. coli</i> , <i>Citrobacter rodentium</i> | Tir | Bacterial receptor located in host membrane, recruits SHP-1 that negatively interferes with signalling (interferes with TRAF6 ubiquitination) | Yan <i>et al.</i> , 2012 |
| | <i>Brucella melitensis</i> , <i>Br. abortus</i> | TcpB/Btp1 | Mimics TIRAP, causing its degradation | Sengupta <i>et al.</i> , 2010; Radhakrishnan <i>et al.</i> , 2009 |
| | <i>E. coli</i> | TcpC | Binds to MyD88 and negatively interferes with signalling | Cirl <i>et al.</i> , 2008 |
| IRAKs | <i>Po. gingivalis</i> | LPS | Induces expression of the inhibitory IRAK-M | Domon <i>et al.</i> , 2008 |
| TRAF | <i>Y. pestis</i> | YopJ | Prevents K63-polyubiquitination of TRAF6 | Sweet <i>et al.</i> , 2007; Zhou <i>et al.</i> , 2005 |
| | <i>Shig. flexneri</i> | OpsI | Deamidates and inactivates the ubiquitin-conjugating enzyme Ubc13 | Sanada <i>et al.</i> , 2012 |
| TAK/TAB | <i>Helicobacter pylori</i> | CagA | Enhances the activity of TAK1 | Lamb <i>et al.</i> , 2009 |
| | <i>E. coli</i> | NleE | Methylates TAB2 and TAB3, thereby inhibiting the UBD binding ability | Zhang <i>et al.</i> , 2011b |
| IKK complex | <i>Y. pseudotuberculosis</i> | YopJ | Acetylates the activation loop of IKK α and IKK β , thereby preventing their phosphorylation-induced activation | Mittal <i>et al.</i> , 2006 |
| | <i>Shig. flexneri</i> | IpaH9.8 | E3 ligase that polyubiquitinates NEMO, resulting in its degradation | Ashida <i>et al.</i> , 2010 |
| | <i>E. coli</i> | NleE | Inhibits IKK phosphorylation and activation | Nadler <i>et al.</i> , 2010 |
| I κ B | <i>L. pneumophila</i> | LegK1 | Ser/Thr-kinase-like protein that phosphorylates I κ B | Ge <i>et al.</i> , 2009 |
| | <i>Shig. flexneri</i> | OspG | Inhibits ubiquitin-conjugating enzymes, thereby preventing ubiquitination of I κ B | Kim <i>et al.</i> , 2005 |
| | <i>Sal. typhimurium</i> | AvrA | Deubiquitinates I κ B | Ye <i>et al.</i> , 2007 |
| | <i>Y. pseudotuberculosis</i> , <i>Chlamydia trachomatis</i> | YopJ, ChlaDub1 | Deubiquitinates K48-I κ B | Zhou <i>et al.</i> , 2005; Le Negrate <i>et al.</i> , 2008 |

Table 1. cont.

| Targeted step in the pathway | Bacterial species | Bacterial molecule/effector | Mechanism of action | Reference |
|------------------------------|-----------------------------------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| NFκB | <i>Shig. flexneri</i> | OspF | Dual phosphatase that prevents histone H3 phosphorylation in a subset of κB responsive genes | Arbibe <i>et al.</i> , 2007 |
| | <i>E. coli</i> | NleE | Prevents nuclear translocation of p65 and c-Rel | Newton <i>et al.</i> , 2010 |
| | <i>Bo. pertussis</i> <i>Ba. thetaiotaomicron</i> | BopN | Promotes translocation of repressive p50 PPAR-γ mediated export of RelA to cytoplasm | Nagamatsu <i>et al.</i> , 2009 Kelly <i>et al.</i> , 2004 |
| | <i>E. coli</i> | NleC | Degrades p65 | Yen <i>et al.</i> , 2010; Pearson <i>et al.</i> , 2011; Baruch <i>et al.</i> , 2011; Mühlen <i>et al.</i> , 2011 |
| | <i>E. coli</i> | NleH1 | Inhibits IKKβ-mediated phosphorylation of RPS3 Ser209, thereby preventing its nuclear translocation | Wan <i>et al.</i> , 2011 |
| | <i>E. coli</i> | NleC | Degrades the p300 co-activator | Shames <i>et al.</i> , 2011 |

activated receptor-γ (PPAR-γ) in the host cells. Presence of *Ba. thetaiotaomicron*, in addition to *Salmonella enteritidis*, triggered a physical interaction between RelA and PPAR-γ, and nuclear export of both proteins. The interaction was dependent on the C-terminal ligand-binding domain of PPAR-γ while the transport was independent of exportin-1. However, the precise mechanism of PPAR-γ/RelA export is unknown (Kelly *et al.*, 2004).

Recently, ribosomal protein S3 (RPS3) was found to be an essential part of certain native NFκB complexes. RPS3 interacts with p65 and increases the DNA binding ability of the complex (Wan *et al.*, 2007). RPS3 directs the transcriptional complex to certain cognate κB sites, resulting in induced expression of immunoglobulin κ light chain in B cells, and cell proliferation and cytokine secretion in T cells (Wan & Lenardo, 2010; Wan *et al.*, 2007). Activation of the NFκB pathway can induce IKKβ-mediated phosphorylation of RPS3 at serine 209. This phosphorylation enhances the association of RPS3 with importin-α, which is required for nuclear translocation of RPS3 (Wan *et al.*, 2011). The virulence protein NleH1 from EHEC and EPEC interacts with and inhibits the phosphorylation of RPS3 both *in vitro* and *in vivo* thereby blocking RPS3 nuclear translocation and function (Fig. 2). (Gao *et al.*, 2009; Wan *et al.*, 2011) Gnotobiotic piglets infected with Δ*nleH1* strain had a robust inflammatory response, but reduced bacterial colonization and little diarrhoea, suggesting that NleH1 might be an advantage for the bacterium in colonization and transmission (Pham *et al.*, 2012).

NFκB can influence transcription by recruitment of co-activators such as the host acetyltransferase p300/CBP. These co-activators can enhance the transcriptional activity of NFκB by various mechanisms. The co-activators can acetylate histone tails causing them to lose their electrostatic interaction with DNA, which is an advantage for

transcription. Additionally, p300 can regulate the NFκB protein RelA/p65 by acetylation (Chen *et al.*, 2002). Therefore, it may not be so surprising that microbes also try to attack co-activators as a strategy for immune evasion. The zinc-dependent protease NleC from *E. coli* has recently also been found to interact with p300, causing its degradation. Overexpression or depletion of p300 in mammalian cells resulted in increased or reduced IL-8 expression, respectively (Shames *et al.*, 2011). The NleC-induced degradation of p300 may therefore contribute to an altered cytokine response.

Conclusions and future perspectives

Microbes are recognized and eradicated by the host immune system. In order to either survive as commensals or to establish successful infections, several microbes have evolved a wide range of immune evasion strategies. Here, we have provided examples showing that bacteria can evade almost every single step in the canonical NFκB pathway. Some bacteria encode several independent effectors that can block the PRR–NFκB pathway on multiple levels to ensure silencing of immune responses (Table 1). Others produce individual effectors that target several steps in the pathway, thereby overriding the PAMP-induced signalling (Table 1). The mechanism of action varies. Many effectors mimic the function of well-known host proteins, while others act as decoys for receptors or specific inhibitors of the canonical pathway, or the bacteria may by its presence be able to induce expression of endogenous inhibitors of the pathway within the host cell (Biswas *et al.*, 2011). In any case, the net result is hijacking of the host TLR–NFκB signalling.

Hijacking the TLR–NFκB pathway can be beneficial for commensals when establishing their niche in the host

(Neish *et al.*, 2000). Bacterial interference with host signalling can also be beneficial for infection. During acute infection, the hijacking results in increased bacterial reproduction within the host before recognition by immune cells. This strategy is seen among pathogenic strains of, among others, *E. coli*, *Shigella*, *Klebsiella pneumoniae* and *Salmonella* (Cirl *et al.*, 2008; Ashida *et al.*, 2010; March *et al.*, 2011; Newman *et al.*, 2006). Many of the aforementioned pathogens infect the intestine, and the integrity of the intestinal layer is of importance for the health and survival of the host. The presence of bacterial effectors that negatively interfere with inflammation may protect the bacteria from the immune response, but may also protect the host intestine from inflammatory destruction of the tissue. By this mechanism, the bacteria increase their chance of spread through diarrhoea (Kim *et al.*, 2005; Le Negrato *et al.*, 2008; Pham *et al.*, 2012). Hijacking the TLR–NF κ B pathway may also be beneficial during chronic infection. In periodontitis, the expression of CXCR4 is elevated, and targeted by the Pg-fimbriae of *Po. gingivalis* resulting in inhibition of TLR2-induced NF κ B activation. Moreover, *Po. gingivalis* induces expression of the endogenous negative regulator IRAK-M. The two mechanisms for escaping the pathway may contribute to chronic infection, when the bacteria remain persistently (Hajishengallis *et al.*, 2008). Other bacteria specifically target immune cells, and here their influence on signalling pathways may be of particular importance. Stimulated dendritic cells will mature and express surface molecules that stimulate T-cells in the lymph node. *Brucella abortus* can invade and replicate within dendritic cells, where the *Br. abortus*-encoded Btp1 inhibits NF κ B activation and disturbs DC maturation, resulting in reduced ability to stimulate T-cells (Salcedo *et al.*, 2008). The net outcome is beneficial to the bacteria.

Most of the intracellular effectors shown in Table 1 are produced by Gram-negative bacteria which inject the effector molecule(s) into the host cell through their type III or IV secretion system (Newton *et al.*, 2010; Lamb *et al.*, 2009; Ge *et al.*, 2009). However, patients with MyD88- or IRAK4-deficiencies are highly susceptible to infections by Gram-positive bacteria, while they have normal resistance to Gram-negative bacteria and other pathogens (von Bernuth *et al.*, 2012). Moreover, mice deficient of or with modified versions of TLR2, TIRAP, MyD88 and/or IRAK4 were more susceptible to infection with *Staph. aureus*, *En. faecium* and/or *Strep. pneumoniae* infection (Takeuchi *et al.*, 2000; Hoebe *et al.*, 2005; Deshmukh *et al.*, 2009; Leendertse *et al.*, 2008; Pennini *et al.*, 2013; Albiger *et al.*, 2005). This suggests that the MyD88/IRAK-4 pathway is of particular importance for defence against Gram-positive organisms, and it might not be a surprise if Gram-positive organisms also target this pathway as an immune evasion strategy. However, this remains to be elucidated.

Increased understanding of bacterial immune evasion mechanisms targeting TLR–NF κ B signalling may be of great medical interest. Activation of the canonical NF κ B pathway is important in defence against invading

micro-organisms. However, aberrant activation of the signalling can be part of inflammatory and autoimmune diseases or cancer (Jeong & Lee, 2011; Han & Ulevitch, 2005; Zhu & Mohan, 2010). Microbes have evolved strategies to avoid activation of the pathway and the bacterial effectors may themselves, or in modified versions, have therapeutic potential against the above-mentioned diseases. The first example involves *Propionibacterium acnes*, which is thought to be involved in creating inflammation in acne vulgaris, a skin disease especially common among teenagers. A recent *in vitro* study showed that the *En. faecalis* bacteriocin CBT-SL5 negatively interfered with *Pr. acnes*-induced inflammation of keratinocytes (Lee *et al.*, 2008). The molecular mechanism was not addressed, but the net effect was that a bacterial molecule inhibited inflammation. Continuing and future testing may result in a useful anti-inflammatory agent. Another example involves peptides that mimic the BB-loop in the TIR domain of MyD88. Such peptides were found to attenuate staphylococcal enterotoxin B-induced pro-inflammatory cytokine production and toxicity in mice (Kissner *et al.*, 2011). Instead of using purified bacterial protein as an anti-inflammatory agent, another strategy may be to create bacteria that have an anti-inflammatory effect on certain host tissues. This strategy was tested in a mouse model of inflammatory bowel disease. Here, oral administration of lactic acid bacteria expressing the anti-inflammatory elafin had an anti-inflammatory effect in the intestine (Motta *et al.*, 2012). In summary, increased understanding of bacterial immune evasion strategies may provide new options for creating drugs to use in treatment of inflammatory diseases in the future.

Acknowledgements

Our work is supported by grants from the Norwegian Research Council and the Odd Berg Foundation.

References

- Abeyta, M., Hardy, G. G. & Yother, J. (2003). Genetic alteration of capsule type but not PspA type affects accessibility of surface-bound complement and surface antigens of *Streptococcus pneumoniae*. *Infect Immun* 71, 218–225.
- Albiger, B., Sandgren, A., Katsuragi, H., Meyer-Hoffert, U., Beiter, K., Wartha, F., Hornef, M., Normark, S. & Normark, B. H. (2005). Myeloid differentiation factor 88-dependent signalling controls bacterial growth during colonization and systemic pneumococcal disease in mice. *Cell Microbiol* 7, 1603–1615.
- Arbibe, L., Kim, D. W., Batsche, E., Pedron, T., Mateescu, B., Muchardt, C., Parsot, C. & Sansonetti, P. J. (2007). An injected bacterial effector targets chromatin access for transcription factor NF κ B to alter transcription of host genes involved in immune responses. *Nat Immunol* 8, 47–56.
- Ashida, H., Kim, M., Schmidt-Supprian, M., Ma, A., Ogawa, M. & Sasakawa, C. (2010). A bacterial E3 ubiquitin ligase IpaH9.8 targets NEMO/IKK γ to dampen the host NF κ B-mediated inflammatory response. *Nat Cell Biol* 12, 66–73, 1–9.

- Bardoel, B. W., Vos, R., Bouman, T., Aerts, P. C., Bestebroer, J., Huizinga, E. G., Brondijk, T. H., van Strijp, J. A. & de Haas, C. J. (2012). Evasion of Toll-like receptor 2 activation by staphylococcal superantigen-like protein 3. *J Mol Med (Berl)* **90**, 1109–1120.
- Barrow, A. D. & Trowsdale, J. (2006). You say ITAM and I say ITIM, let's call the whole thing off: the ambiguity of immunoreceptor signalling. *Eur J Immunol* **36**, 1646–1653.
- Baruch, K., Gur-Arie, L., Nadler, C., Koby, S., Yerushalmi, G., Ben-Neriah, Y., Yogev, O., Shaulian, E., Guttman, C. & other authors (2011). Metalloprotease type III effectors that specifically cleave JNK and NF- κ B. *EMBO J* **30**, 221–231.
- Bestebroer, J., De Haas, C. J. & Van Strijp, J. A. (2010). How microorganisms avoid phagocyte attraction. *FEMS Microbiol Rev* **34**, 395–414.
- Biswas, A., Wilmanski, J., Forsman, H., Hrcir, T., Hao, L., Tlaskalova-Hogenova, H. & Kobayashi, K. S. (2011). Negative regulation of Toll-like receptor signaling plays an essential role in homeostasis of the intestine. *Eur J Immunol* **41**, 182–194.
- Burns, K., Janssens, S., Brissoni, B., Olivos, N., Beyaert, R. & Tschopp, J. (2003). Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J Exp Med* **197**, 263–268.
- Carpenter, S. & O'Neill, L. A. (2007). How important are Toll-like receptors for antimicrobial responses? *Cell Microbiol* **9**, 1891–1901.
- Casanova, J. L., Abel, L. & 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.
- Chen, Z. J. (2005). Ubiquitin signalling in the NF- κ B pathway. *Nat Cell Biol* **7**, 758–765.
- Chen, L. F., Mu, Y. & Greene, W. C. (2002). Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF- κ B. *EMBO J* **21**, 6539–6548.
- Cigana, C., Curcuru, L., Leone, M. R., Ieranò, T., Lorè, N. I., Bianconi, I., Silipo, A., Cozzolino, F., Lanzetta, R. & other authors (2009). *Pseudomonas aeruginosa* exploits lipid A and muropeptides modification as a strategy to lower innate immunity during cystic fibrosis lung infection. *PLoS ONE* **4**, e8439.
- Cirl, C., Wieser, A., Yadav, M., Duerr, S., Schubert, S., Fischer, H., Stappert, D., Wantia, N., Rodriguez, N. & other authors (2008). Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. *Nat Med* **14**, 399–406.
- Collier-Hyams, L. S., Zeng, H., Sun, J., Tomlinson, A. D., Bao, Z. Q., Chen, H., Madara, J. L., Orth, K. & Neish, A. S. (2002). Cutting edge: *Salmonella* AvrA effector inhibits the key proinflammatory, anti-apoptotic NF- κ B pathway. *J Immunol* **169**, 2846–2850.
- Deshmukh, H. S., Hamburger, J. B., Ahn, S. H., McCafferty, D. G., Yang, S. R. & Fowler, V. G., Jr (2009). Critical role of NOD2 in regulating the immune response to *Staphylococcus aureus*. *Infect Immun* **77**, 1376–1382.
- Domon, H., Honda, T., Oda, T., Yoshie, H. & Yamazaki, K. (2008). Early and preferential induction of IL-1 receptor-associated kinase-M in THP-1 cells by LPS derived from *Porphyromonas gingivalis*. *J Leukoc Biol* **83**, 672–679.
- Fraser, J. D. & Proft, T. (2008). The bacterial superantigen and superantigen-like proteins. *Immunol Rev* **225**, 226–243.
- Gao, X., Wan, F., Mateo, K., Callegari, E., Wang, D., Deng, W., Puente, J., Li, F., Chaussee, M. S. & other authors (2009). Bacterial effector binding to ribosomal protein s3 subverts NF- κ B function. *PLoS Pathog* **5**, e1000708.
- Ge, J., Xu, H., Li, T., Zhou, Y., Zhang, Z., Li, S., Liu, L. & Shao, F. (2009). A *Legionella* type IV effector activates the NF- κ B pathway by phosphorylating the I κ B family of inhibitors. *Proc Natl Acad Sci U S A* **106**, 13725–13730.
- Gunn, J. S. & Ernst, R. K. (2007). The structure and function of *Francisella* lipopolysaccharide. *Ann N Y Acad Sci* **1105**, 202–218.
- Hajishengallis, G., Wang, M., Liang, S., Triantafilou, M. & Triantafilou, K. (2008). Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function. *Proc Natl Acad Sci U S A* **105**, 13532–13537.
- Han, J. & Ulevitch, R. J. (2005). Limiting inflammatory responses during activation of innate immunity. *Nat Immunol* **6**, 1198–1205.
- Hoebel, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., Sovath, S., Shamel, L., Hartung, T. & other authors (2005). CD36 is a sensor of diacylglycerides. *Nature* **433**, 523–527.
- Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Takeda, K. & Akira, S. (1999). Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. *J Immunol* **162**, 3749–3752.
- Huang, T. T., Kudo, N., Yoshida, M. & Miyamoto, S. (2000). A nuclear export signal in the N-terminal regulatory domain of I κ B α controls cytoplasmic localization of inactive NF- κ B/I κ B α complexes. *Proc Natl Acad Sci U S A* **97**, 1014–1019.
- Janssens, S., Burns, K., Tschopp, J. & Beyaert, R. (2002). Regulation of interleukin-1- and lipopolysaccharide-induced NF- κ B activation by alternative splicing of MyD88. *Curr Biol* **12**, 467–471.
- Jeong, E. & Lee, J. Y. (2011). Intrinsic and extrinsic regulation of innate immune receptors. *Yonsei Med J* **52**, 379–392.
- Jiang, X. & Chen, Z. J. (2012). The role of ubiquitylation in immune defence and pathogen evasion. *Nat Rev Immunol* **12**, 35–48.
- Kawasaki, K., Ernst, R. K. & Miller, S. I. (2004). 3-O-deacylation of lipid A by PagL, a PhoP/PhoQ-regulated deacylase of *Salmonella typhimurium*, modulates signaling through Toll-like receptor 4. *J Biol Chem* **279**, 20044–20048.
- Kelly, D., Campbell, J. I., King, T. P., Grant, G., Jansson, E. A., Coutts, A. G., Pettersson, S. & Conway, S. (2004). Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR- γ and RelA. *Nat Immunol* **5**, 104–112.
- Kersse, K., Bertrand, M. J., Lamkanfi, M. & Vandenabeele, P. (2011). NOD-like receptors and the innate immune system: coping with danger, damage and death. *Cytokine Growth Factor Rev* **22**, 257–276.
- Kim, D. W., Lenzen, G., Page, A. L., Legrain, P., Sansonetti, P. J. & Parsot, C. (2005). The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc Natl Acad Sci U S A* **102**, 14046–14051.
- Kissner, T. L., Moisan, L., Mann, E., Alam, S., Ruthel, G., Ulrich, R. G., Rebek, M., Rebek, J., Jr & Saikh, K. U. (2011). A small molecule that mimics the BB-loop in the Toll interleukin-1 (IL-1) receptor domain of MyD88 attenuates staphylococcal enterotoxin B-induced pro-inflammatory cytokine production and toxicity in mice. *J Biol Chem* **286**, 31385–31396.
- Kumar, H., Kawai, T. & Akira, S. (2009). Pathogen recognition in the innate immune response. *Biochem J* **420**, 1–16.
- Lamb, A., Yang, X. D., Tsang, Y. H., Li, J. D., Higashi, H., Hatakeyama, M., Peek, R. M., Blanke, S. R. & Chen, L. F. (2009). *Helicobacter pylori* CagA activates NF- κ B by targeting TAK1 for TRAF6-mediated Lys 63 ubiquitination. *EMBO Rep* **10**, 1242–1249.
- Le Negrato, G., Faustin, B., Welsh, K., Loeffler, M., Krajewska, M., Hasegawa, P., Mukherjee, S., Orth, K., Krajewski, S. & other authors (2008). *Salmonella* secreted factor L deubiquitinase of *Salmonella*

- typhimurium* inhibits NF- κ B, suppresses I κ B α ubiquitination and modulates innate immune responses. *J Immunol* **180**, 5045–5056.
- Lee, M. S. & Kim, Y. J. (2007). Signaling pathways downstream of pattern-recognition receptors and their cross talk. *Annu Rev Biochem* **76**, 447–480.
- Lee, Y. J., Choi, H. J., Kang, T. W., Kim, H. O., Chung, M. J. & Park, Y. M. (2008). CBT-SL5, a bacteriocin from *Enterococcus faecalis*, suppresses the expression of interleukin-8 induced by *Propionibacterium acnes* in cultured human keratinocytes. *J Microbiol Biotechnol* **18**, 1308–1316.
- Leendertse, M., Willems, R. J., Giebelen, I. A., van den Pangaart, P. S., Wiersinga, W. J., de Vos, A. F., Florquin, S., Bonten, M. J. & van der Poll, T. (2008). TLR2-dependent MyD88 signaling contributes to early host defense in murine *Enterococcus faecium* peritonitis. *J Immunol* **180**, 4865–4874.
- Li, Q. & Verma, I. M. (2002). NF-kappaB regulation in the immune system. *Nat Rev Immunol* **2**, 725–734.
- Li, C., Zienkiewicz, J. & Hawiger, J. (2005). Interactive sites in the MyD88 Toll/interleukin (IL) 1 receptor domain responsible for coupling to the IL1 β signaling pathway. *J Biol Chem* **280**, 26152–26159.
- Liu, M., Haenssler, E., Uehara, T., Losick, V. P., Park, J. T. & Isberg, R. R. (2012). The *Legionella pneumophila* EnhC protein interferes with immunostimulatory muramyl peptide production to evade innate immunity. *Cell Host Microbe* **12**, 166–176.
- Losick, V. P., Haenssler, E., Moy, M. Y. & Isberg, R. R. (2010). LnaB: a *Legionella pneumophila* activator of NF- κ B. *Cell Microbiol* **12**, 1083–1097.
- Madan-Lala, R., Peixoto, K. V., Re, F. & Rengarajan, J. (2011). *Mycobacterium tuberculosis* Hip1 dampens macrophage proinflammatory responses by limiting toll-like receptor 2 activation. *Infect Immun* **79**, 4828–4838.
- March, C., Moranta, D., Regueiro, V., Llobet, E., Tomás, A., Garmendia, J. & Bengoechea, J. A. (2011). *Klebsiella pneumoniae* outer membrane protein A is required to prevent the activation of airway epithelial cells. *J Biol Chem* **286**, 9956–9967.
- Mittal, R., Peak-Chew, S. Y. & McMahon, H. T. (2006). Acetylation of MEK2 and I κ B kinase (IKK) activation loop residues by YopJ inhibits signaling. *Proc Natl Acad Sci U S A* **103**, 18574–18579.
- Motta, J. P., Bermúdez-Humarán, L. G., Deraison, C., Martin, L., Rolland, C., Rousset, P., Boue, J., Dietrich, G., Chapman, K. & other authors (2012). Food-grade bacteria expressing elafin protect against inflammation and restore colon homeostasis. *Sci Transl Med* **4**, 158ra144.
- Mühlen, S., Ruchaud-Sparagano, M. H. & Kenny, B. (2011). Proteasome-independent degradation of canonical NF κ B complex components by the NleC protein of pathogenic *Escherichia coli*. *J Biol Chem* **286**, 5100–5107.
- Nadler, C., Baruch, K., Kobi, S., Mills, E., Haviv, G., Farago, M., Alkalay, I., Bartfeld, S., Meyer, T. F. & other authors (2010). The type III secretion effector NleE inhibits NF-kappaB activation. *PLoS Pathog* **6**, e10000743.
- Nagamatsu, K., Kuwae, A., Konaka, T., Nagai, S., Yoshida, S., Eguchi, M., Watanabe, M., Mimuro, H., Koyasu, S. & Abe, A. (2009). *Bordetella* evades the host immune system by inducing IL-10 through a type III effector, BopN. *J Exp Med* **206**, 3073–3088.
- Nakayama, M., Underhill, D. M., Petersen, T. W., Li, B., Kitamura, T., Takai, T. & Aderem, A. (2007). Paired Ig-like receptors bind to bacteria and shape TLR-mediated cytokine production. *J Immunol* **178**, 4250–4259.
- Nanra, J. S., Buitrago, S. M., Crawford, S., Ng, J., Fink, P. S., Hawkins, J., Scully, I. L., McNeil, L. K., Aste-Amézaga, J. M. & other authors (2012). Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. *Hum Vaccin Immunother* **9**.
- Neish, A. S., Gewirtz, A. T., Zeng, H., Young, A. N., Hobert, M. E., Karmali, V., Rao, A. S. & Madara, J. L. (2000). Prokaryotic regulation of epithelial responses by inhibition of I κ B- α ubiquitination. *Science* **289**, 1560–1563.
- Nestle, F. O., Di Meglio, P., Qin, J. Z. & Nickoloff, B. J. (2009). Skin immune sentinels in health and disease. *Nat Rev Immunol* **9**, 679–691.
- Newman, R. M., Salunkhe, P., Godzik, A. & 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.
- Newton, K. & Dixit, V. M. (2012). Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol* **4**, a006049.
- Newton, H. J., Pearson, J. S., Badea, L., Kelly, M., Lucas, M., Holloway, G., Wagstaff, K. M., Dunstone, M. A., Sloan, J. & other authors (2010). The type III effectors NleE and NleB from enteropathogenic *E. coli* and OspZ from *Shigella* block nuclear translocation of NF- κ B p65. *PLoS Pathog* **6**, e1000898.
- Nish, S. & Medzhitov, R. (2011). Host defense pathways: role of redundancy and compensation in infectious disease phenotypes. *Immunity* **34**, 629–636.
- O'Neill, L. A. & Bowie, A. G. (2007). The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* **7**, 353–364.
- O'Riordan, K. & Lee, J. C. (2004). *Staphylococcus aureus* capsular polysaccharides. *Clin Microbiol Rev* **17**, 218–234.
- Oeckinghaus, A., Hayden, M. S. & Ghosh, S. (2011). Crosstalk in NF- κ B signaling pathways. *Nat Immunol* **12**, 695–708.
- Pearson, J. S., Riedmaier, P., Marchès, O., Frankel, G. & Hartland, E. L. (2011). A type III effector protease NleC from enteropathogenic *Escherichia coli* targets NF- κ B for degradation. *Mol Microbiol* **80**, 219–230.
- Pennini, M. E., Perkins, D. J., Salazar, A. M., Lipsky, M. & Vogel, S. N. (2013). Complete dependence on IRAK4 kinase activity in TLR2, but not TLR4, signaling pathways underlies decreased cytokine production and increased susceptibility to *Streptococcus pneumoniae* infection in IRAK4 kinase-inactive mice. *J Immunol* **190**, 307–316.
- Pham, T. H., Gao, X., Tsai, K., Olsen, R., Wan, F. & Hardwidge, P. R. (2012). Functional differences and interactions between the *Escherichia coli* type III secretion system effectors NleH1 and NleH2. *Infect Immun* **80**, 2133–2140.
- Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M. & other authors (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085–2088.
- Radhakrishnan, G. K., Yu, Q., Harms, J. S. & Splitter, G. A. (2009). *Brucella* TIR domain-containing protein mimics properties of the Toll-like receptor adaptor protein TIRAP. *J Biol Chem* **284**, 9892–9898.
- Salcedo, S. P., Marchesini, M. I., Lelouard, H., Fugier, E., Jolly, G., Balor, S., Muller, A., Lapaque, N., Demaria, O. & other authors (2008). *Brucella* control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. *PLoS Pathog* **4**, e21.
- Sanada, T., Kim, M., Mimuro, H., Suzuki, M., Ogawa, M., Oyama, A., Ashida, H., Kobayashi, T., Koyama, T. & other authors (2012). The *Shigella flexneri* effector OspI deamidates UBC13 to dampen the inflammatory response. *Nature* **483**, 623–626.
- Sengupta, D., Koblansky, A., Gaines, J., Brown, T., West, A. P., Zhang, D., Nishikawa, T., Park, S. G., Roop, R. M., II & Ghosh, S. (2010). Subversion of innate immune responses by *Brucella* through

- the targeted degradation of the TLR signaling adapter, MAL. *J Immunol* **184**, 956–964.
- Shames, S. R., Bhavsar, A. P., Croxen, M. A., Law, R. J., Mak, S. H., Deng, W., Li, Y., Bidshari, R., de Hoog, C. L. & other authors (2011). The pathogenic *Escherichia coli* type III secreted protease NleC degrades the host acetyltransferase p300. *Cell Microbiol* **13**, 1542–1557.
- Slevogt, H., Zabel, S., Opitz, B., Hocke, A., Eitel, J., N'guessan, P. D., Lucka, L., Riesbeck, K., Zimmermann, W. & other authors (2008). CEACAM1 inhibits Toll-like receptor 2-triggered antibacterial responses of human pulmonary epithelial cells. *Nat Immunol* **9**, 1270–1278.
- Spear, A. M., Loman, N. J., Atkins, H. S. & Pallen, M. J. (2009). Microbial TIR domains: not necessarily agents of subversion? *Trends Microbiol* **17**, 393–398.
- Spear, A. M., Rana, R., Jenner, D. C., Flick-Smith, H. C., Oyston, P. C., Simpson, P., Matthews, S., Byrne, B. & Atkins, H. S. (2012). A TIR domain protein from *Yersinia pestis* interacts with mammalian IL-1/TLR pathways but does not play a central role in the virulence of *Y. pestis* in a mouse model of bubonic plague. *Microbiology* **158**, 1593–1606.
- Suhir, H. & Etzioni, A. (2010). The role of Toll-like receptor signaling in human immunodeficiencies. *Clin Rev Allergy Immunol* **38**, 11–19.
- Sun, S. C. (2012). The noncanonical NF- κ B pathway. *Immunol Rev* **246**, 125–140.
- Sweet, C. R., Conlon, J., Golenbock, D. T., Goguen, J. & Silverman, N. (2007). YopJ targets TRAF proteins to inhibit TLR-mediated NF- κ B, MAPK and IRF3 signal transduction. *Cell Microbiol* **9**, 2700–2715.
- Takeda, K. & Akira, S. (2004). TLR signaling pathways. *Semin Immunol* **16**, 3–9.
- Takeuchi, O., Hoshino, K. & Akira, S. (2000). Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J Immunol* **165**, 5392–5396.
- Thurlow, L. R., Thomas, V. C., Fleming, S. D. & Hancock, L. E. (2009). *Enterococcus faecalis* capsular polysaccharide serotypes C and D and their contributions to host innate immune evasion. *Infect Immun* **77**, 5551–5557.
- Turvey, S. E. & Hawn, T. R. (2006). Towards subtlety: understanding the role of Toll-like receptor signaling in susceptibility to human infections. *Clin Immunol* **120**, 1–9.
- Underhill, D. M., Ozinsky, A., Hajjar, A. M., Stevens, A., Wilson, C. B., Bassetti, M. & Aderem, A. (1999). The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* **401**, 811–815.
- van der Windt, G. J., Blok, D. C., Hoogerwerf, J. J., Lammers, A. J., de Vos, A. F., Van't Veer, C., Florquin, S., Kobayashi, K. S., Flavell, R. A. & van der Poll, T. (2012). Interleukin 1 receptor-associated kinase m impairs host defense during pneumococcal pneumonia. *J Infect Dis* **205**, 1849–1857.
- Veldkamp, K. E. & van Strijp, J. A. (2009). Innate immune evasion by staphylococci. *Adv Exp Med Biol* **666**, 19–31.
- von Bernuth, H., Picard, C., Puel, A. & Casanova, J. L. (2012). Experimental and natural infections in MyD88- and IRAK-4-deficient mice and humans. *Eur J Immunol* **42**, 3126–3135.
- Wan, F. & Lenardo, M. J. (2010). The nuclear signaling of NF- κ B: current knowledge, new insights, and future perspectives. *Cell Res* **20**, 24–33.
- Wan, F., Anderson, D. E., Barnitz, R. A., Snow, A., Bidere, N., Zheng, L., Hegde, V., Lam, L. T., Staudt, L. M. & other authors (2007). Ribosomal protein S3: a KH domain subunit in NF- κ B complexes that mediates selective gene regulation. *Cell* **131**, 927–939.
- Wan, F., Weaver, A., Gao, X., Bern, M., Hardwidge, P. R. & Lenardo, M. J. (2011). IKK β phosphorylation regulates RPS3 nuclear translocation and NF- κ B function during infection with *Escherichia coli* strain O157:H7. *Nat Immunol* **12**, 335–343.
- Wanke, I., Steffen, H., Christ, C., Krismer, B., Götz, F., Peschel, A., Schaller, M. & Schitteck, B. (2011). Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *J Invest Dermatol* **131**, 382–390.
- Watters, T. M., Kenny, E. F. & O'Neill, L. A. (2007). Structure, function and regulation of the Toll/IL-1 receptor adaptor proteins. *Immunol Cell Biol* **85**, 411–419.
- Wolfert, M. A., Roychowdhury, A. & Boons, G. J. (2007). Modification of the structure of peptidoglycan is a strategy to avoid detection by nucleotide-binding oligomerization domain protein 1. *Infect Immun* **75**, 706–713.
- Xavier, R. J. & Podolsky, D. K. (2000). Microbiology. How to get along—friendly microbes in a hostile world. *Science* **289**, 1483–1484.
- Xu, Y., Tao, X., Shen, B., Horng, T., Medzhitov, R., Manley, J. L. & Tong, L. (2000). Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* **408**, 111–115.
- Yadav, M., Zhang, J., Fischer, H., Huang, W., Lutay, N., Cirl, C., Lum, J., Miethke, T. & Svanborg, C. (2010). Inhibition of TIR domain signaling by TcpC: MyD88-dependent and independent effects on *Escherichia coli* virulence. *PLoS Pathog* **6**, e1001120.
- Yan, D., Wang, X., Luo, L., Cao, X. & Ge, B. (2012). Inhibition of TLR signaling by a bacterial protein containing immunoreceptor tyrosine-based inhibitory motifs. *Nat Immunol* **13**, 1063–1071.
- Ye, Z., Petrof, E. O., Boone, D., Claud, E. C. & Sun, J. (2007). *Salmonella* effector AvrA regulation of colonic epithelial cell inflammation by deubiquitination. *Am J Pathol* **171**, 882–892.
- Yen, H., Ooka, T., Iguchi, A., Hayashi, T., Sugimoto, N. & Tobe, T. (2010). NleC, a type III secretion protease, compromises NF- κ B activation by targeting p65/RelA. *PLoS Pathog* **6**, e1001231.
- Yokoyama, R., Itoh, S., Kamoshida, G., Takii, T., Fujii, S., Tsuji, T. & Onozaki, K. (2012). Staphylococcal superantigen-like protein 3 binds to the Toll-like receptor 2 extracellular domain and inhibits cytokine production induced by *Staphylococcus aureus*, cell wall component, or lipopeptides in murine macrophages. *Infect Immun* **80**, 2816–2825.
- Zhang, Q., Zmasek, C. M., Cai, X. & Godzik, A. (2011a). TIR domain-containing adaptor SARM is a late addition to the ongoing microbe-host dialog. *Dev Comp Immunol* **35**, 461–468.
- Zhang, L., Ding, X., Cui, J., Xu, H., Chen, J., Gong, Y. N., Hu, L., Zhou, Y., Ge, J. & other authors (2011b). Cysteine methylation disrupts ubiquitin-chain sensing in NF- κ B activation. *Nature* **481**, 204–208.
- Zhou, H., Monack, D. M., Kayagaki, N., Wertz, I., Yin, J., Wolf, B. & Dixit, V. M. (2005). *Yersinia* virulence factor YopJ acts as a deubiquitinase to inhibit NF- κ B activation. *J Exp Med* **202**, 1327–1332.
- Zhu, J. & Mohan, C. (2010). Toll-like receptor signaling pathways—therapeutic opportunities. *Mediators Inflamm* **2010**, 781235.

Edited by: S. Spiro