REVIEW ARTICLE

# Staphylococcus aureus modulation of innate immune responses through Toll-like (TLR), (NOD)-like (NLR) and C-type lectin (CLR) receptors

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**One sentence summary:** Microbial pathogens have a perpetual co-evolutionary interaction with the innate immune system of their respective hosts. This review outlines the multitude of mechanisms utilized by the leading human bacterial pathogen *Staphylococcus aureus* to modulate and evade proinflammatory responses induced by Toll-like (TLR), (NOD)-like (NLR) and C-type lectin (CLR) receptors.

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## ABSTRACT

Early recognition of pathogens by the innate immune system is crucial for bacterial clearance. Many pattern recognition receptors (PRRs) such as Toll-like (TLRs) and (NOD)-like (NLRs) receptors have been implicated in initial sensing of bacterial components. The intracellular signaling cascades triggered by these receptors result in transcriptional upregulation of inflammatory pathways. Although this step is crucial for bacterial elimination, it is also associated with the potential for substantial immunopathology, which underscores the need for tight control of inflammatory responses. The leading human bacterial pathogen *Staphylococcus aureus* expresses over 100 virulence factors that exert numerous effects upon host cells. In this manner, the pathogen seeks to avoid host recognition or perturb PRR-induced innate immune responses to allow optimal survival in the host. These immune system interactions may result in enhanced bacterial proliferation but also provoke systemic cytokine responses associated with sepsis. This review summarizes recent findings on the various mechanisms applied by *S. aureus* to modulate or interfere with inflammatory responses through PRRs. Detailed understanding of these complex interactions can provide new insights toward future immune-stimulatory therapeutics against infection or immunomodulatory therapeutics to suppress or correct dysregulated inflammation.

**Keywords:** *Staphylococcus aureus*; pattern recognition receptors; inflammatory responses; evasion; virulence; biotherapeutic

## INTRODUCTION

Classical inflammation can be triggered by a variety of stimuli such as microbial pathogens or tissue damage, which increase energy expenditure and robustly activate various proinflammatory signaling and effector pathways (Han and Ulevitch 2005). The inflammatory response is rapid and characterized by enhancement of vascular flow and permeability, allowing leakage of serum components into the tissue microenvironment.

Extravasation of immune cells to the affected tissue normally promotes healing and regeneration. However, overreaction or failure to appropriately shut down the acute inflammatory response may lead to an uncontrolled 'cytokine storm', which can be detrimental to the host. This can be precipitated by a wide range of infectious (e.g. septic shock) as well as noninfectious diseases (e.g. asthma or inflammatory bowel disease) (Collins 1996; Tisoncik *et al.* 2012). Thus, the immune system must carefully fine-tune the level of inflammation to clear the pathogenic threat while avoiding additional tissue damage (Weiss *et al.* 2008).

Innate immune responses are composed of multiple dynamic defense systems, which together play the sentinel role in early recognition of invading pathogens, deployment of generalized antimicrobial factors, induction of proinflammatory responses, recruitment of phagocytic cells and activation of acquired immunity (Medzhitov and Janeway 2000). Acquired immunity subsequently aids in clearance of pathogens with a higher level specificity and the generation of immunological memory (Iwasaki and Medzhitov 2004). The innate immune response is mediated through several factors, including recognition of conserved microbial structures termed 'pathogen-associated molecular patterns' (PAMPs) via a broad array of germline-encoded pattern recognition receptors (PRRs). In addition, PRRs can recognize endogenous molecules produced by damaged cells or tissues, called damage-associated molecular patterns (Mogensen 2009). PRRs comprise Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), cytosolic DNA sensors and retinoic acid-inducible (RIG)-I-like receptors (RLRs) (Xia *et al.* 2016; Ori, Murase and Kawai 2017). RLRs comprise retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), and probable ATP-dependent RNA helicase DHX58 (LGP2), which coordinate antiviral responses (Loo and Gale 2011) and thus will not be further discussed in this review.

A distinctive pattern of subcellular distribution for each of the different PRR classes provides functional redundancy and synergy towards recognition of an extensive range of PAMPs derived from a diverse array of potential pathogens including bacteria, fungi, protozoa and viruses. PRR-based pathogen detection results in activation of several signaling pathways that converge on key transcription factors such as nuclear factor-kappa B (NF-$\kappa$B), activator protein 1 (AP1) and interferon (IFN) regulatory factors. The cumulative effect of PRR signaling is the differential expression of key proinflammatory as well as antiinflammatory genes (Takeuchi and Akira 2010). Activation of PRRs-mediated inflammatory responses must be tightly controlled: too weak of a response heightens host susceptibility to infection; on the other hand, an overactive response may promote lethal systemic inflammation, autoimmunity, or the development of acute or chronic inflammatory diseases (O'Neill, Bryant and Doyle 2009).

*Staphylococcus aureus* is among the most medically important bacterial pathogens that persistently colonizes the anterior nares of 30% of healthy individuals. In addition, this bacterium can cause a wide range of serious human infections such as bacteremia, endocarditis, pneumonia, skin and wound infections and deep tissue abscesses (Weidenmaier, Goerke and Wolz 2012; Krismer *et al.* 2017). Genome-wide analysis on *S. aureus* suggests downregulation of virulence genes during colonization and upregulation of them in the course of infection (Novick 2003). The treatment of *S. aureus* infections is complicated by the increasing number of antibiotic-resistant strains (DeLeo *et al.* 2010; Tong

*et al.* 2015) and the induction of hemodynamic changes that may prove resistant to therapeutic modalities (Polat *et al.* 2017). Moreover, when pyogenic infection arises, a robust host inflammatory response is induced both locally and systemically, often termed sepsis syndrome. The peak cytokine response in Gram-positive pyogenic infections like *S. aureus* occurs 50–75 h after the challenge (Opal and Cohen 1999). Despite significant advancement in treatment modalities, sepsis-induced mortality rate is 20%–30% in Gram-positive bacterial sepsis and can be as high as 70%–90% when shock and multiorgan failure are present (Polat *et al.* 2017).

In the struggle to avoid immune clearance, the pathogen deploys several mechanisms to subvert the fast-acting innate immune responses through interference with PRR recognition, restraining complement deposition or activation and delaying neutrophil recruitment (Foster 2005; Rooijakkers, van Kessel and van Strijp 2005; Kim *et al.* 2012; Foster *et al.* 2014). The understanding of staphylococcal immune evasion of both innate and adaptive immunity has been an area of intense research. In this light, initial recognition of *S. aureus* by PRRs is pivotal in setting the course of the host-pathogen interaction and orchestrating the accompanying immune responses. Our aim in this review is to highlight and discuss recent findings on staphylococcal strategies to modulate host innate immune and inflammatory responses via PRRs (Table 1), a multifaceted interaction that brings into play a large array of the pathogen's evasion factors and virulence determinants.

## Toll-like receptor signaling

Human TLRs (TLR1-TLR10) are transmembrane glycoproteins composed of extracellular leucine-rich repeats (LRRs), a transmembrane domain and a cytoplasmic tail containing a Toll/IL-1 receptor (TIR) domain. Each TLR can recognize a distinct set of PAMPs (Takeda and Akira 2004; Akira, Uematsu and Takeuchi 2006) either through direct interaction, e.g. TLR1/TLR2, TLR3 and TLR9 (Jin *et al.* 2007; Latz *et al.* 2007; Liu *et al.* 2008), or indirectly via an accessory PAMP-binding molecule, e.g. interaction between LPS and the MD2-TLR4 complex (Jin *et al.* 2007). TLRs are differentially expressed and distributed in different cell types. Some TLRs are displayed on the cell surface and specialized in recognition of PAMPs and endogenous misplaced proteins, whereas other TLRs are mainly localized in intracellular compartments and recognize nucleic acids or other intracellular PAMPs (Iwasaki and Medzhitov 2004; Mogensen 2009; Kawai and Akira 2010) (Fig. 1). TLR expression can be inducible, e.g. in keratinocytes, or constitutive, e.g. in antigen-presenting cells (APCs) (Mogensen 2009). Binding of the cognate ligand (PAMP or DAMP) to the extracellular leucine-rich repeats (LRRs) induces TLR homo- or heterodimerization. TLR2, for example, is functionally active as a heterodimer in cooperation with TLR1 or TLR6 to achieve specificity and discriminate subtle differences in the repertoire of bacterial lipoproteins (reviewed in Farhat *et al.* (2008)). Dimerization subsequently results in recruitment of TIR-containing adaptor protein(s) to the intracellular TIR domain of the receptor (Takeda and Akira 2004; O'Neill and Bowie 2007; Song and Lee 2012). Five different TIR-containing adaptors are known to be recruited depending on the stimuli and TLRs involved (Akira, Takeda and Kaisho 2001; Takeda and Akira 2004): Myeloid differentiation factor 88 (MyD88), TIR-associated protein (TIRAP, also known as MyD88 adaptor-like protein (Mal)), TIR-domain-containing adaptor protein including interferon-$\beta$

**Table 1.** TLRs, NLRs and CLRs involved *in S. aureus* recognition.

| Receptor-family | Specific receptor | *S. aureus*—derived ligand | Reference |
|---|---|---|---|
| TLR | TLR2 | LTA, lipoprotein | Schwandner *et al.* (1999), Takeuchi *et al.* (1999), Iwaki *et al.* (2002), Sabroe *et al.* (2003) |
| | TLR2-TLR1/TLR6 | Lipopeptide, lipoprotein | Ozinsky *et al.* (2000), Morr *et al.* (2002), Nishiya and DeFranco (2004), Travassos *et al.* (2004) |
| | TLR2-CD14 | Peptidoglycan, LTA | Schroder *et al.* (2003) |
| | TLR2-CD36 | Diacylglycerides, LTA | Hoebe *et al.* (2005) |
| | TLR2- Asialo-GM1 | Unknown (probably a surface molecule regulated by Agr) | Ratner *et al.* (2001), Soong *et al.* (2004) |
| | TLR8 | *S. aureus* RNA | Bergstrom *et al.* (2015) |
| | TLR9 | *S. aureus* unmethylated CPG DNA | Parker and Prince (2012) |
| NLR | NOD2 | Peptidoglycan (MDP) | Volz *et al.* (2010) |
| CLRs | MBL | LTA | Polotsky *et al.* (1996) |
| | L-ficolin | LTA | Lynch *et al.* (2004) |
| | SP-A | LTA, CWG | Lawson and Reid (2000) |

LTA: lipoteichoic acid; CPG: cytosine-phosphate-guanosine; MDP: muramyl dipeptide; MBL: mannose binding lectin; CWG: cell wall glycopolymer; SP: surfactant protein.

(TRIF), TRIF-related adaptor molecule (TRAM), or sterile adaptor $\alpha$- and armadillo-motif-containing protein (SARM).

TIRAP has been distinctly involved in bridging MyD88 to TLR2 and TLR4 (Akira, Takeda and Kaisho 2001; Takeda and Akira 2004). MyD88 has a modular structure composed of an N-terminal death domain (DD), an intermediate domain (ID) and a C-terminal TIR domain (TIR) (Takeda and Akira 2004). The three domains of MyD88 are associated with interleukin-1 receptor-associated kinase 4 (IRAK4), IRAK1 and the TIR domain of TLRs, respectively. Binding of IRAK4 mediates the phosphorylation of IRAK1 (Janssens *et al.* 2003; Takeda and Akira 2004) and recruitment of tumor necrosis factor receptor-associated factor 6 (TRAF6) to the receptor complex. Phosphorylated IRAK1 and TRAF6 dissociate from the receptor complex and associate with another complex composed of transforming growth factor-$\beta$-activated kinase-1 (TAK1), TAK1 binding protein 1 (TAB1) and TAB2. This assembly, in turn, triggers activation of two distinct signaling pathways via the inhibitor of nuclear factor-$\kappa$B (IKK) complex and the mitogen-activated protein kinases (MAPKs) (Akira, Uematsu and Takeuchi 2006; Kawai and Akira 2007; Kawai and Akira 2010). IKK complex formation, composed of IKK$\alpha$, IKK$\beta$ and IKK$\gamma$/NEMO, is the bottleneck for multiple pathways ultimately leading to NF-$\kappa$B activation, and thus important in regulating immune and inflammatory signaling pathways (Hacker and Karin 2006; Mogensen 2009; Kawai and Akira 2010) (Fig. 2). The TAK1 complex functions as a MAPK kinase kinase kinase (MAP3K), initiating a cascade involving phosphorylation of MAP kinase kinase (MAP2K) followed by activation of the MAP kinase (MAPK) subfamilies p38 and Jun-N-terminal kinase (JNK). The MAPK extracellular signal-regulated kinase (ERK) is also activated by TAK1 via the IKK complex. Stimulation of these pathways results in activation factor-1 (AP-1) induction (Ninomiya-Tsuji *et al.* 1999; Kawai and Akira 2011). The TLR induced-activation of NF-$\kappa$B and AP-1 play a critical role in the induction of inflammatory and innate immune responses, by regulating the expression of a vast number of cytokines, chemokines and antimicrobial effectors (Li and Verma 2002; Lawrence 2009; Xia *et al.* 2016).

## The importance of TLR-mediated signaling in bacterial clearance

The primary staphylococcal PRR located on the plasma membrane is TLR2, which recognizes bacteria-derived PAMPs such as lipoteichoic acid (LTA) and lipoproteins (Schwandner *et al.* 1999; Iwaki *et al.* 2002; Schroder *et al.* 2003; Dziarski and Gupta 2005). TLR2 is expressed by different cells involved in the inflammatory response such as neutrophils, monocytes/macrophages, dendritic cells, mast cells and astrocytes (reviewed in Fournier and Philpott (2005)). The dual ligand specificity of TLR2 is determined by its dimerization partner. The formation of TLR2-TLR6 heterodimers is initiated by diacyl lipopeptides from Gram-positive bacteria, including *S. aureus*, (Takeuchi *et al.* 2001), while triacyl lipopeptides from Gram-negative bacteria induce the formation of heterodimers with TLR1 (Takeuchi *et al.* 2002) (Fig. 1).

TLR2 plays an important role in protection against sepsis caused by *S. aureus* strains that produce phenol-soluble modulin (PSM) (Hanzelmann *et al.* 2016). In addition, a critical role for TLR2 signaling in innate immunity is corroborated by the impact of naturally occurring mutations or polymorphisms in the receptor or downstream components involved in TLR2 signaling, increasing the risk of developing pyogenic bacterial infections. For instance, TLR2- (Takeuchi, Hoshino and Akira 2000; Gonzalez-Zorn *et al.* 2005; Hoebe *et al.* 2005; Strunk *et al.* 2010) and MyD88-deficient (Takeuchi, Hoshino and Akira 2000) mice are predisposed to *S. aureus* infection and MRSA nasal colonization. Also children carrying mutations in genes encoding TLR- signaling components, such as MyD88, or IRAK4 (Picard *et al.* 2010; von Bernuth *et al.* 2012) or children with the TLR2 polymorphisms Arg753Gln, are at high risk of developing infections with *S. aureus* and other Gram-positive bacterial pathogens (Carpenter and O'Neill 2007).

## TLRs, NLRs and CLRs localization and their ligands
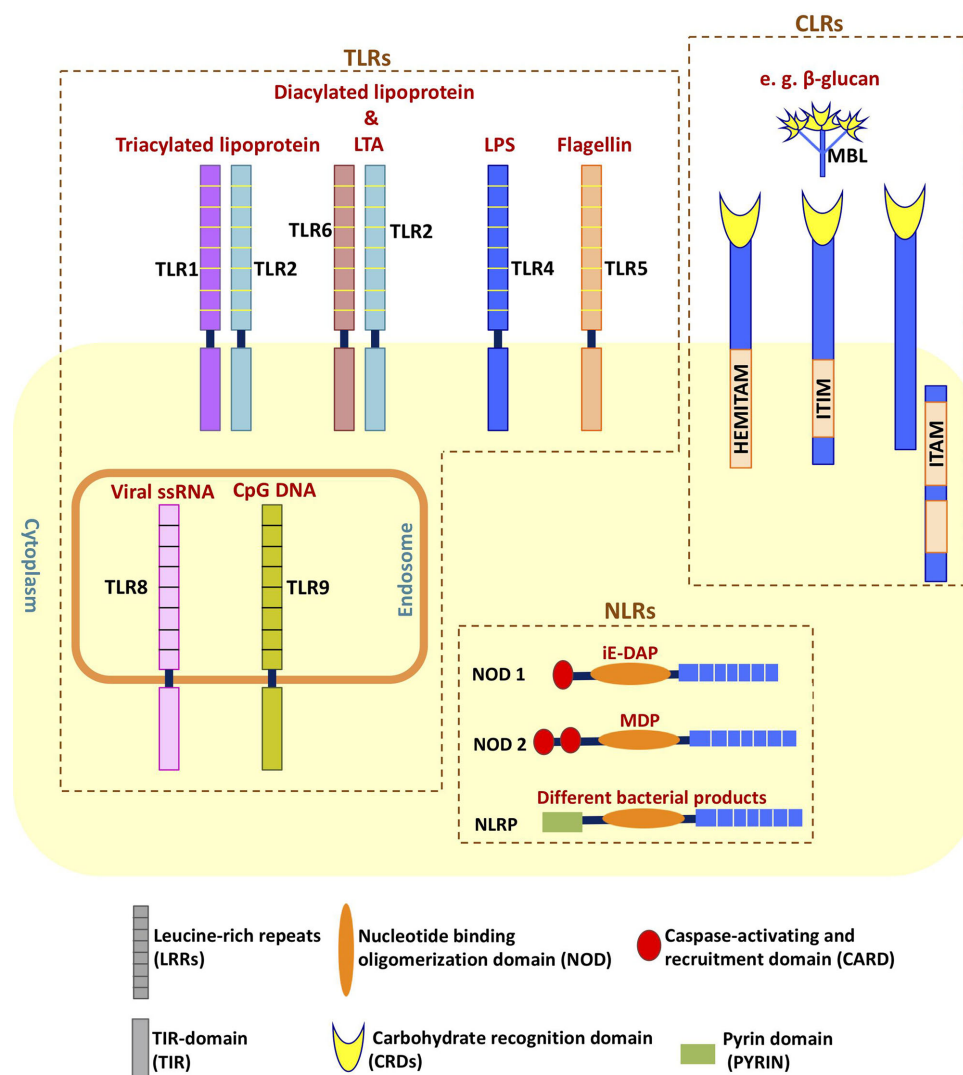


**Figure 1.** *Toll-like receptor (TLR), NOD-like receptor (NLR) and C-type lectin receptor (CLR) localization and example of ligand.* TLRs can be located at plasma membrane and at endosomes, while NLRs are localized in cytoplasm. CLRs include soluble molecules such as MBL, and plasma membrane located receptors. See text for details. LTA: Lipoteichoic acid; LPS: Lipopolysaccharide; ssRNA: Single-stranded ribonucleic acid; iE-DAP: D-γ-glutamyl-meso-DAP dipeptide; MDP: Muramyl dipeptide; MBL: mannose binding lectin; ITIM: immunoreceptor tyrosine-based inhibition motifs; ITAM: immune-receptor tyrosine-based activation motif.

## Downregulation of TLR2-mediated NF-$\kappa$B activation by *S. aureus*

*Staphylococcus aureus* is detected by the host through its multiple PAMPs, which leads to the release of proinflammatory mediators and antimicrobial peptides (AMPs) through PRR signaling and inflammasome activation (reviewed in Fournier and Philpott (2005)). TLR2 activates the immune cells in response to its agonists, bacterial lipoproteins (Takeuchi, Hoshino and Akira 2000; Hashimoto *et al.* 2006). *Staphylococcus aureus* PSMs could influence the activation of TLR2 since the protein supports mobilizing and release of lipoprotein (Hanzelmann *et al.* 2016). Notably, *S. aureus* deficient in lipoprotein synthesis lose their capability to activate this receptor (Stoll *et al.* 2005). In addition, the pathogen dampens appropriate TLR2 activation by at least

three distinct mechanisms: inhibition of heterodimer formation, structural mimicry of the TIR domain and activation of inhibitory receptor pathways. These three mechanisms are described below.

*Evasion of initial recognition and heterodimer formation*
The role of staphylococcal superantigen-like proteins (SSLs) in pathogenesis has been proven in a large number of studies (Baker *et al.* 2007; Bestebroer *et al.* 2007; de Haas *et al.* 2009; Walenkamp *et al.* 2009; Itoh *et al.* 2010a,b; Patel *et al.* 2010; Walenkamp *et al.* 2010; Koymans *et al.* 2017). The SSL family consist of 14 different members (SSL1-SSL14), out of which SSL1-SSL11 and SSL12-SSL14 proteins are encoded by genes located on staphylococcal pathogenicity island 2 (SaPI2) and

## Stimuli-induced TLR2- and NOD2 mediated signaling
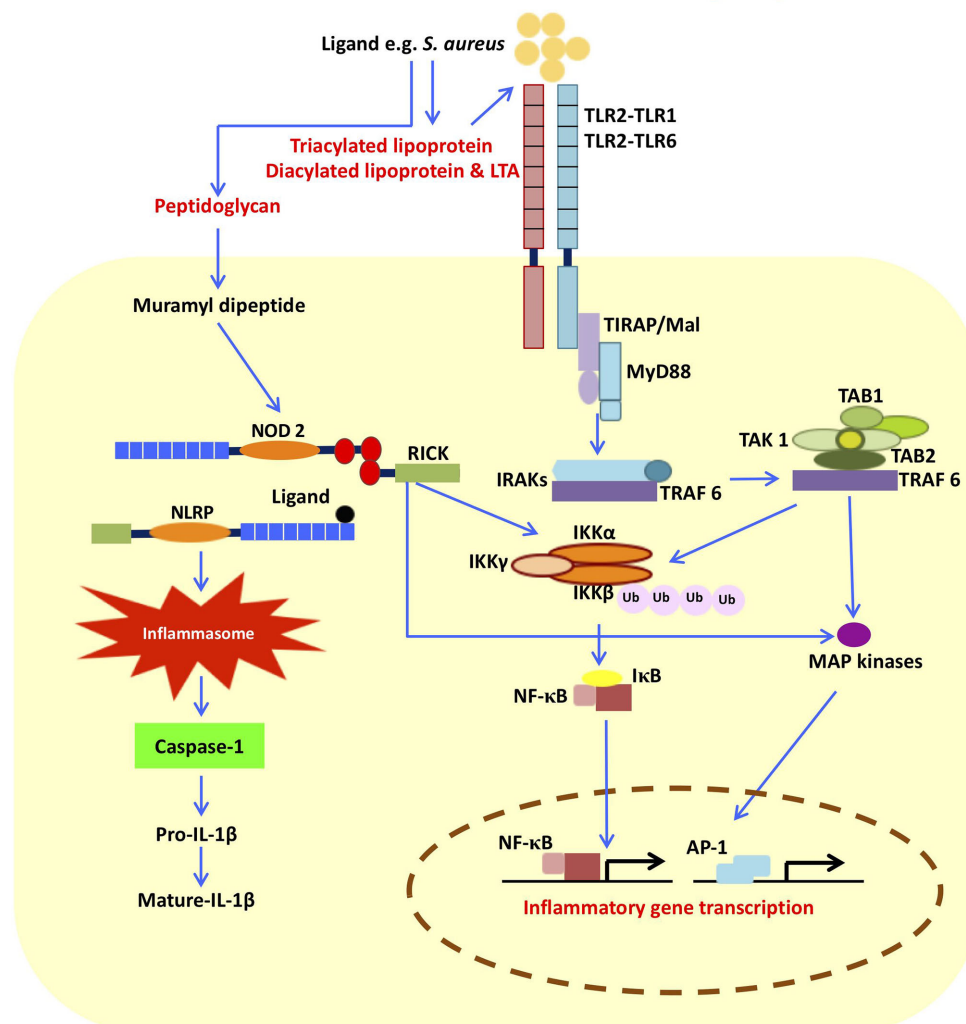


**Figure 2.** *Stimuli-induced TLR2- and NOD2- mediated signaling.* The TLR- (e.g. TLR2) pathway is mainly mediated through MyD88. TIRAP is a sorting adaptor used by TLR2 and TLR4. MyD88 recruits IRAKs and TRAF6 and ultimately induces pro-inflammatory responses through activation of NF-$\kappa$B and AP-1. NOD- (e.g. NOD2) induced NF-$\kappa$B activation is mediated through RICK by triggering the K63-type polyubiquitination of IKK$\beta$. The cooperation between NODs and NLRPs induces inflammasome formation resulting in production of mature IL-1$\beta$. MyD88: myeloid differentiation factor-88, TIRAP: TIR associated protein, IRAK: IL-1 receptor associated kinase, TRAF: tumor necrosis factor receptor-associated factor, TAK1: growth factor-$\beta$-activated kinase-1, TAB: TAK1 binding protein 1, IKK: IkappaB kinase, MAPK: mitogen activated protein kinase, NF-$\kappa$B: nuclear factor kappa B, AP-1: activator protein 1, RICK: CARD-containing serine/threonine kinase.

immune evasion cluster 2 (IEC2), respectively (Fitzgerald *et al.* 2003; Jongerius *et al.* 2007). Among these, SSL3, and to a lesser extent SSL4, negatively interfere with TLR2 recognition, with a net outcome of suppressing chemokine (e.g. IL-8) production by HEK cells explicitly expressing TLR1/2 and TLR2/6 dimers (Bardoel *et al.* 2012). SSL3 has also been shown to block TLR2-mediated secretion of tumor necrosis factor (TNF) in murine macrophages (Yokoyama *et al.* 2012). In the latter case, SSL3 blocked binding of bacterial lipopeptides to the extracellular domain of TLR2 and in the case of ligand binding, prevented the formation of TLR2–TLR1 and TLR2–TLR6 heterodimers (Koymans *et al.* 2015).

*Evasion through structural mimicry*
Structural mimicry of host proteins is an important mechanism by which pathogens manipulate host immune responses (Stebbins and Galan 2001), including TLR-mediated signaling through PAMP recognition (reviewed in Johannessen *et al.* (2014)). Bac-

terial TIR-homologous proteins have been identified in a wide range of Gram-negative bacteria, and more recently, in Gram-positive species. Members of the bacterial TIR-homologous protein family are typically 230-310 amino acids in size, wherein the conserved TIR domain, 150-200 amino acids in length, is localized at the C-terminus or N-terminus, while the remaining protein sequence shows a high degree of variation (reviewed in Rana *et al.* (2013)). The majority of investigations on bacterial TIR proteins have demonstrated that these proteins negatively interfere with TLR signaling pathways and consequently inhibit NF-$\kappa$B activation (Newman *et al.* 2006; Low *et al.* 2007; Cirl *et al.* 2008; Radhakrishnan *et al.* 2009; Rana *et al.* 2011; Askarian *et al.* 2014; Kraemer *et al.* 2014; Zou *et al.* 2014). However, on one occasion, the bacterial TIR protein resulted in activation of same pathway (Patterson *et al.* 2014). Recently a TIR-containing protein (TirS) was identified in the genome of S. *aureus* strain MSSA476 and found to be localized at the mobile genetic element 'staphylococcal chromosomal cassettes' (SCC)$_{476}$. TirS

exerted a specific inhibitory effect against stimulus-induced TLR2-mediated NF-$\kappa$B activation, JNK phosphorylation, and production of proinflammatory cytokines. Furthermore, a comparison of pathogenicity of the MSSA476 wild-type strain vs. its isogenic Δ*tirS* mutant in an intravenous mouse infection model revealed that presence of TirS increased the bacterial load in multiple organs (Askarian *et al.* 2014). Later, TirS was reported to be present in 12% of *S. aureus* (both MSSA and MRSA) strains, and found to downregulate NF-$\kappa$B pathway through inhibition of not only TLR2, but also TLR4, TLR5 and TLR9 (Patot *et al.* 2017). TirS in these *S. aureus* strains was localized within SCC together with genes encoding fusidic acid resistance and, in some cases, genes providing resistance to methicillin. The characterization of these SSC elements links two critical clinco-pathological features of contemporary staphylococcal infection, immune evasion and antibiotic resistance, since exposure to fusidic acid increased TirS expression (Patot *et al.* 2017). TirS has also been described in an animal isolate of *S. aureus* (Patterson *et al.* 2014). The only other Gram-positive bacterial TIR protein known to date was identified in *Entereococcus faecalis*, where it is localized in a genomic region of phage origin, which might suggest horizontal gene transfer (Kraemer *et al.* 2014; Zou *et al.* 2014).

### Manipulation of TLRs by activating inhibitory receptors

Inhibitory immune receptors negatively interfere with signal transduction induced by activating receptors. Many inhibitory receptors have specific intracellular sequence motifs, like immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Activation of ITIM-bearing receptors results in phosphorylation of ITIMs and subsequent recruitment of mediators, which causes them to negatively regulate inflammatory signals from activating receptors such as TLRs (van Avondt, van Sorge and Meyaard 2015). Murine paired Ig-like receptor (PIR)-B is identified as a receptor for *S. aureus* LTA. PIR-B has ITIMs in the cytoplasmic domain and negatively regulates inflammatory cytokine production in response to heat-killed *S. aureus in vitro*. Mice that are deficient in PIR-B (*Pirb-/-*) have reduced bacterial load in blood, increased clearance of *S. aureus* and survive longer than wild-type mice after infection, showing the adverse effect of PIR-B activation for *S. aureus* clearance (Nakayama *et al.* 2007, 2012).

## Potential biotherapeutic implications of interference with TLR2 signaling

Insights into the negative regulation of TLR signaling by microbes such as *S. aureus* may open new avenues to develop treatments of uncontrolled inflammation.

Therapeutic interference with anomalous immune responses may in theory be possible. The therapeutic potential of microbial TIR domain proteins may be utilized in a highly specific way as they come in a wide variety and have fine differences in their action on TLR signaling (Rana *et al.* 2013). Derived from a *vaccinia* virus A46R protein, VIPER was generated as a potent inhibitor of TLR4-mediated responses (Lysakova-Devine *et al.* 2010). Cell-penetrating inhibitory peptides are targeting TLR adapter-receptor interactions were based on the BB loop region of the TIR domain and the intermediate domain within MyD88 (reviewed in Fekonja, Avbelj and Jerala (2012)). Interestingly their action could be enhanced by applying concepts discovered in microbial TIR domain proteins. Since coiled–coiled mediated TIR domain dimerization is a method exerted by bacterial TIR domain proteins (Newman *et al.* 2006), this strategy can be utilized to improve wide-spectrum TLR inhibitors

(reviewed in Fekonja, Avbelj and Jerala (2012)). Thus research on eukaryotic and bacterial TIR domains or modified variants of them together is a valuable source of templates and strategies for drug design to downregulate an overactive innate immune response. Taken together, these drug development strategies suggest that antiinflammatory bacterial proteins targeting TLRs could be co-opted prevent, dampen or treat inflammatory diseases.

TLR2 also has a unique role in cancer progression and metastasis (Huang *et al.* 2008), and the potential for anti-TLR2 antibody or TLR2 antagonist therapy in cancer immunotherapy has been suggested (Yang *et al.* 2009, 2012). Negative regulators of TLRs have been identified and characterized (Kondo, Kawai and Akira 2012) and interference with TLR2 could also be of therapeutic interest for certain infections. For example, the addition of anti-TLR2 antibody to monocytes infected with *Mycobacterium avium* subsp. *paratuberculosis* enhances phagosome maturation and intracellular bacterial clearance (Weiss *et al.* 2008). Moreover, immune-neutralization of TLR2 function with a monoclonal antibody against the extracellular domain of TLR2 was reported to induce a modulatory response to *S. aureus* during acute infection *in vivo* (Meng *et al.* 2004). In contrast, pretreatment of animals with corticosteroids did not reduce mortality during *S. aureus* infection (Opal and Cohen 1999) and anticytokine therapies had a detrimental effect in Gram-positive sepsis (Opal and Cohen 1999; Tracey and Abraham 1999). In all cases, the collaborative activity among different PRRs must be considered in the development of therapeutic strategies against inflammation, cancer and/or infection.

## NOD like receptor signaling

The nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) are a family of intracellular/cytosolic PRRs, consisting of more than 20 members in humans. NLRs recognize PAMPs and endogenous molecules introduced into host cell cytosol (Moreira and Zamboni 2012). These receptors are classified to five different subfamilies based on N-terminal structural characteristics and include NLRA (A for acidic transactivation domain), NLRB (B for baculovirus inhibitor of apoptosis protein repeat), NLRC (C for caspase recruitment domain CARD), NLRP (P for pyrin domain) and NLRX (X for no significant homology). The activation of NLRs can result in transcriptional activation (e.g. NLRA), autophagy (e.g. NLRCs and NLRX), inflammasome formation (e.g. NLRPs and NLRB) and/or signal transduction (e.g. NLRCs (Motta *et al.* 2015)). In the following, NLRPs and NLRCs are described in more detail.

Activation of NLRPs induces the assembly of a multiprotein platform called the inflammasome, triggers activation of caspase-1 to promote maturation and secretion of caspase 1-dependent inflammatory cytokines such as IL-1$\beta$ and IL-18, and ultimately initiates programmed cellular pyroptosis (Munoz-Planillo *et al.* 2009; Schroder and Tschopp 2010), an extremely rapid form of programmed host cell death. Pyroptosis leads to the sudden release of numerous host molecules that further amplify inflammation (Aachoui *et al.* 2013). In general, NLRP3 can be activated upon sensing a wide range of stress signals, e.g. lysosomal damage, reactive oxygen species (ROS), the efflux of potassium, and pathogen-derived molecules (Willingham *et al.* 2007; Schroder and Tschopp 2010). The genetic background, at least in mice, can influence the level of macrophage pyroptosis, which in turn can affect the progression of *S. aureus* infection (Accarias *et al.* 2015).

The NLRCs (NOD1 and NOD2) play key roles in both bacterial and non-bacterial infections. Their structures consist of a C-terminal LRR domain, which mediates protein–protein interaction, a central nucleotide-binding domain, and either a single or two N-terminal CARD domain(s). Activation of NOD1 and NOD2 is triggered by two different peptidoglycan motifs: D-glutamyl-meso-diaminopimelic acid (iE-DAP) of Gram-negative bacteria or muramyl dipeptide (MDP), which is the minimal bioactive structure of peptidoglycan in Gram-positive bacteria (Strober *et al.* 2006; Moreira and Zamboni 2012; Oviedo-Boyso, Bravo-Patino and Baizabal-Aguirre 2014) (Fig. 1). Recombinant NOD2 binds MDP with high affinity, confirming a direct interaction and that NOD2 functions as a cytosolic PRR (Grimes *et al.* 2012). Monomeric peptidoglycan fragments of *S. aureus* induce NOD2 activation (Volz *et al.* 2010). Furthermore, the *S. aureus* α-hemolysin, a pore-forming cytotoxin, facilitates NOD2-dependent recognition of *S. aureus* during cutaneous infection (Hruz *et al.* 2009).

Activation of NF-$\kappa$B signaling pathways through NOD1 and NOD2 is associated with recruitment of a CARD-containing serine/threonine kinase called receptor-interacting *serine/threonine* protein *kinase* 2 (RICK, also called RIP2 and RIPK2), which interacts with the CARD domain(s) of NOD1 and NOD2. Homotopic CARD–CARD interaction is achieved through recruitment of E3 ubiquitin ligase TRAF6 to RICK, which results in a series of ubiquitination-dependent events and ultimately activation of the TAK1 complex. Activated TAK1 triggers K63-type polyubiquitination of IKK$\beta$ and subsequently NF-$\kappa$B activation (Ogura *et al.* 2001; Strober *et al.* 2006; Hasegawa *et al.* 2008; Moreira and Zamboni 2012) (Fig. 2). Furthermore, activation of other signaling pathways, including the p38, ERK and JNK through NODs-RICK interaction, has been reported. NODs can also interact with NLRPs (e.g. NLRP1, NLRP3 and NLRP12) and result in caspase-1 activation and maturation of L-1$\beta$ and IL-18 (Strober *et al.* 2006; Moreira and Zamboni 2012 and references therein) (Fig. 2).

## Consequence of *S. aureus* activation of NLRs

The importance of NOD2 in induction of various cytokines, e.g. IL-1$\beta$ and IL-6, and/or antimicrobial peptides, e.g. $\beta$-defensin, in host defense against *S. aureus* infection, has been demonstrated (Deshmukh *et al.* 2009; Hruz *et al.* 2009). For instance, NOD2-deficient mice show increased susceptibility and mortality upon intraperitoneal *S. aureus* infection. This susceptibility phenotype was associated with failure of neutrophil phagocytosis, high bacterial load in several organs and magnified production of Th-1 derived cytokines (Deshmukh *et al.* 2009). Subcutaneous infection of NOD2-deficient mice with *S. aureus* resulted in large skin ulcerations, impaired bacterial clearance and delayed proinflammatory responses (Hruz *et al.* 2009). Other data demonstrate that while NOD2 is not essential for bacterial clearance in experimental *S. aureus* pneumonia, it contributes significantly to the regulation of the corresponding lung inflammation during infection. Notably, pulmonary lesions and inflammation, which are a critical factor during the recovery of host from pneumonia, are less severe in NOD2-deficient mice (Kapetanovic *et al.* 2010). Involvement of NOD2 in the initiation of host inflammatory responses in resident brain cells infected with *S. aureus* has also been reported (Liu, Chauhan and Marriott 2010). In addition, mutations in the *NOD2* gene are associated with the development of clinically important chronic inflammatory diseases, such as an injured intestinal epithelial barrier in Crohn's disease (Abreu *et al.* 2002; Cuthbert *et al.* 2002).

Although *S. aureus* is commonly regarded as an extracellular bacterium, it can internalize and invade several types of non-professional phagocytes, including human keratinocytes (Kintarak *et al.* 2004). NOD2 is functional in keratinocytes (Voss *et al.* 2006) and its expression is elevated in the presence of several PAMPs (Kobayashi *et al.* 2009) and during *S. aureus* infection (Roth *et al.* 2014). *Staphylococcus aureus*-induced NOD2 expression is also found in mammary glands during bacterial mastitis (Wang *et al.* 2015). Hence, these data indicate a likely role of NOD2 in intracellular recognition of *S. aureus*. Recently, a contribution of NOD2 in keratinocytes in mediating *S. aureus*-induced IL-17C expression was identified (Roth *et al.* 2014). IL-17C is a member of IL-17 cytokine family, which plays a prominent role in the regulation of epithelial immune responses (Xie *et al.* 2012). Patients suffering from atopic dermatitis (AD), a chronically relapsing inflammatory skin disease, are often colonized with *S. aureus* (Ong 2006). Notably, an association between *NOD2* polymorphism and AD has been identified (Kabesch *et al.* 2003; Macaluso *et al.* 2007), suggesting impairment of *S. aureus* recognition among these patients. Additionally, a role of NOD2 in the induction of type I IFN signaling and secretion of IFN-$\beta$ in response to *S. aureus* has been described (Parker *et al.* 2014).

NLRP3 inflammasome activation can be triggered by phagocytosis of *S. aureus* and the following lysosome-mediated degradation of its peptidoglycan. However, *S. aureus* O-acetyl transferase A modifies the peptidoglycan, rendering it more resistant to lysosome-mediated degradation. In this manner, induction of inflammasome is thereby blunted, and the critical IL-1$\beta$-mediated immune response is subverted (Shimada *et al.* 2010). NLRP3 can be triggered by *S. aureus* α-hemolysin in cultured cells *in vitro* and in murine models of pneumonia and skin infection (Craven *et al.* 2009; Munoz-Planillo *et al.* 2009; Cho *et al.* 2012; Kebaier *et al.* 2012). Notably, *S. aureus* α-hemolysin is expressed by the majority of *S. aureus* isolates (Menzies and Kourteva 2000; Haslinger *et al.* 2003). In addition, *S. aureus* β- and γ- hemolysins (Munoz-Planillo *et al.* 2009), Panton–Valentine leukocidin (PVL) (Holzinger *et al.* 2012) and leukocidin A/B (LukAB, also known as LukGH) (Melehani *et al.* 2015) activate the NLRP3 inflammasome in monocytes/macrophages resulting in maturation and secretion of IL-1$\beta$ and IL-18 and induction of necrotic cell death. However, the role of this NLRP3-driven response in immunity against *S. aureus in vivo* remains controversial. The NLRP3-deficient host-signaling pathway is not required for bacterial clearance in a murine pneumonia infection model though inducing necrotic pulmonary injury (Kebaier *et al.* 2012). However, in a murine *S. aureus* skin infection model, induction of IL-1$\beta$ promotes abscess formation and bacterial clearance (Cho *et al.* 2012). An antitoxin platform has been contemplated for a potential antiinfective/antiinflammatory therapy targeting the NLRP3 inflammasome. Immunization of mice with inactivated α-hemolysin or pharmaceutical inhibition of α-hemolysin attenuates the severity of *S. aureus* pneumonia (Ragle and Bubeck Wardenburg 2009; Ragle, Karginov and Bubeck Wardenburg 2010; Hua *et al.* 2014). Recently, nanosponges have been suggested as a detoxification treatment for a variety of injuries and disease caused by pore-forming toxins such as α-hemolysin. These particles mimic red blood cell membrane and absorb different pore-forming toxins regardless of their molecular structures, and blunt inflammatory activation and pathology (Hu *et al.* 2013; Copp *et al.* 2014; Pang *et al.* 2015; Escajadillo *et al.* 2017).

Another member of this family is NLRP7, which can form an inflammasome after recognizing bacterial acylated lipopeptides, once again resulting in caspase-1-dependent IL-1$\beta$

and IL-18 maturation. In one study, activation of NLRP7 by *S. aureus* was shown to diminish bacterial replication inside human macrophages (Khare *et al.* 2012).

## C-type lectin receptors and recognition of *S. aureus*

Conserved microbial carbohydrate structures are recognized by another class of PRRs called C-type lectin receptors (CLRs) and trigger innate and acquired immune responses (reviewed in Sancho and Reis e Sousa (2012); Hoving, Wilson and Brown (2014)). CLRs are widely expressed by different cell types including APCs. The term 'C-type' refers to their ability to recognition carbohydrates in calcium ($Ca^{2+}$)-dependent manner. However, CLRs are able to recognize many non-carbohydrate ligands, such as lipids and proteins, through mechanisms that are not yet fully understood (Drickamer and Fadden 2002; Zelensky and Gready 2005). The lectin activity of these receptors is mediated by conserved carbohydrate-recognition domains (CRDs). Type I and type II CLRs typically carry multiple or a single CRD(s) domain, respectively. In addition, soluble/circulating CLRs exist and include mannose-binding lectin (MBL), which is an oligomeric protein that binds an array of carbohydrate patterns on pathogen surfaces (Zizzari *et al.* 2015) (Fig. 1). The outcome of CLR-microbe engagement depends on the signaling motif. Generally, phosphorylation of cytoplasmic ITIMs leads to downregulation of cellular processes and the recruitment of phosphatases (e.g. SHP-1 and SHP-2), whereas phosphorylation of immune-receptor tyrosine-based activation motifs (ITAMs/hemITAM), results in an induction of kinase cascades (e.g. spleen tyrosine kinase (Syk)) and cellular activation. However, still another group of CLRs does not possess intracellular signaling motifs and signaling cascades proceed independently of kinases and/or phosphatases. Activation of CLRs ultimately triggers phagocytosis and antimicrobial responses (Sancho and Reis e Sousa 2012; Lepenies, Lee and Sonkaria 2013; Hoving, Wilson and Brown 2014).

Staphylococcal evasion factors for CLR recognition have not been described so far. However, *S. aureus* uses diverse strategies to interfere with later stages of the complement cascades that are induced by CLRs among others (Lambris, Ricklin and Geisbrecht 2008; Zipfel and Skerka 2014). The CLRs Clec-60 and Clec-87 play a role during host immune responses against staphylococcal infection in the invertebrate *C. elegans* model system (JebaMercy and Balamurugan 2012). In humans, low MBL levels and/or MBL2 polymorphism are associated with a high susceptibility to *S. aureus* bacteremia (Chong *et al.* 2014). Even though MBL binds LTA of Gram-positive bacteria, a comparative study found that the LTA of *S. aureus,* which lacks terminal sugars, binds only weakly to MBL (Polotsky *et al.* 1996). L-ficolin, whose structure closely resembles MBL, is another soluble LTA-binding lectin that has been proposed as the major staphylococcal complement activating receptor (Lynch *et al.* 2004). Additionally, *S. aureus* LTA and cell wall glycopolymer (CWG) can be recognized by the surfactant proteins A and D, which are collectins, a subgroup of mammalian lectins (Lawson and Reid 2000; van de Wetering *et al.* 2001). MBL is involved in the activation of the lectin pathway (LP) of the complement system (Matsushita *et al.* 2012) and the number of inhibitors triggered by this pathway by *S. aureus* is noteworthy. For instance, *S. aureus* extracellular adherence protein (Eap) blocks activation of the LP and classical pathway (CP) of complement (Woehl *et al.* 2014, 2017). Unique mechanisms of action employed by staphylococcal complement inhibitors have been reviewed in several publications (Lambris, Ricklin and Geisbrecht 2008; Laarman *et al.* 2010; Zecconi and Scali 2013).
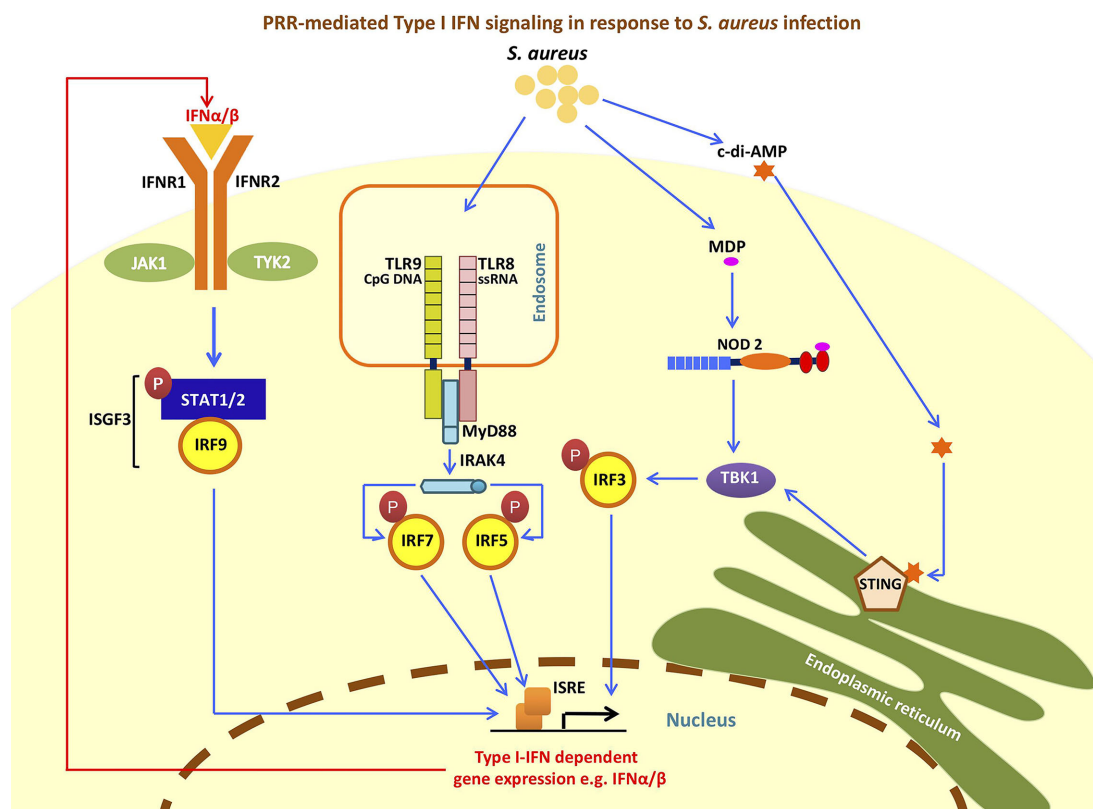
## Type I IFN signaling and recognition of *S. aureus*

Type I IFN signaling was initially identified during viral infections, but the pathway was later demonstrated to be activated by bacteria as well through multiple intracellular and cytosolic receptors (Kovarik *et al.* 2016). More specifically, ligand binding to PRRs, such as TLRs, NLRs, RLRs or DNA sensors, results in activation of interferon regulatory factor (IRF) family of transcription factors, which results in expression of IFN-$\alpha/\beta$. The interferons stimulate cells in an autocrine and paracrine manner (Boxx and Cheng 2016). Mechanistically, IFN-$\alpha/\beta$ binds to its cognate receptor, interferon $\alpha/\beta$ receptor (IFNAR), which leads to activation of hundreds of interferon-stimulated genes (ISGs) through phosphorylation of the homodimeric signal transducer and activation of transcription (STAT)-1 and the heterotrimeric STAT1–STAT2–IRF9 (i.e. interferon-stimulated gene factor (ISGF3) transcription factors) (Decker, Muller and Stockinger 2005; Monroe, McWhirter and Vance 2010) (Fig. 3). The outcome of bacterial infection as a consequence of type I interferon signaling is influenced by many factors including the route and site of infection, bacterial replication and strain-specific virulence factors (Boxx and Cheng 2016).

*Staphylococcus aureus* PAMPs induces type I IFN signaling by various PRRs, depending on the host cell types. Degradation of *S. aureus* in phagosomes can lead to exposure of bacterial deoxycytidylyl-deoxyguanosine dinucleotide (CpG) motifs that trigger a TLR9-mediated enhancement of classical inflammatory cytokines production along with induction of type I interferons (IFNs) (Wolf *et al.* 2011). TLR9 requires an adaptor protein such as MyD88 and enhances mitogen-activated protein kinase and NF-$\kappa$B activation (Wagner 2004). Injection of staphylococcal DNA in a mouse model of cutaneous inflammation causes a robust inflammatory response and arthritis (Deng *et al.* 1999; Molne, Collins and Tarkowski 2003). TLR9 is the pivotal receptor mediating the induction of type I IFN signaling in dendritic cells in response to *S. aureus*. Interestingly, *S. aureus* load was enhanced in the lungs of TLR9$^{-/-}$ mice, which has no defect in recruitment of neutrophils and diminished TNF production (Parker and Prince 2012). Another example is TLR8, which recognizes degradation products of U-rich RNA in the form of uridine and short U-containing oligomers RNA (Heil *et al.* 2004). TLR8 triggers a type I IFN response via an interferon regulatory factor (IRF)-5 signaling axis in primary human monocytes and macrophages. The TLR8-IRF5-type I IFN response in phagocytes is antagonized by TLR2 when the receptor is simultaneously activated by *S. aureus*. Indeed, *S. aureus* lipoprotein activates TLR2 to attenuate TLR8-induced secretion of IFN$\beta$ and IL12 by bacteria degraded within the phagosome (Bergstrom *et al.* 2015). Novel approaches to avoid dysregulation of TLR2/TLR8 signaling may thus improve clinical outcomes in *S. aureus* sepsis.

Type I IFN signaling can also be induced by bacterial second messengers such as cyclic-di-AMP (c-di-AMP) or cyclic-di-GMP (c-di-GMP) (Parvatiyar *et al.* 2012). These second messengers are sensed by the cytosolic DNA sensors DDX4 and IFI16, followed by activation of the transmembrane adaptor at the endoplasmatic reticulum, stimulator of IFN genes (STING) (Boxx and Cheng 2016). Recently, extracellular c-di-AMP was shown to promote *S. aureus* intracellular survival in human monocyte-derived macrophages (Gries *et al.* 2016). However, STING signaling appears to be independent of other DNA sensing pathways such as the TLR9 pathway, although both pathways predominantly use TBK1 (Serine/threonine-protein kinase TBK1)-IRF3 and NF-$\kappa$B to control gene expression (Kumar, Kawai and Akira 2011). Induction of the STING pathway in response to

**Figure 3.** *PRR-mediated Type I IFN signaling in response to S. aureus.* Staphylococcal molecules such as protein A, DNA and RNA are recognized by TLRs, e.g. TLR8 and TLR9, or other types of cytosolic receptors, e.g. NOD2 and STING, resulting in Type I IFN production. NOD2 recognizes the structurally unique MDP, while STING is receptor for e.g. cyclic di-AMP. In either case, an activation of type I IFN signaling occur in a TBK1-IRF3 mediated manner. The TLR-, NOD-, or STING mediated activation of IRF(s) family of transcription factors, results in expression of IFN-$\alpha/\beta$. The type I IFNs, e.g. IFN-$\alpha$ and IFN-$\beta$, bind to their specific receptors IFNRs resulting in phosphorylation and activation JAK1 and TYK2. This leads to phosphorylation of STAT proteins, and consequently, rapid assembly of ISGF-3. The ISGF-3 complex then translocates to the nucleus, where it binds the promoter sequence ISRE. MDP: Muramyl Dipeptides, TBK1: Serine/threonine-protein kinase TBK1, IRF: interferon regulatory factor, JAK1: Janus kinase 1, TYK2: tyrosine kinase 2, STAT: signal transducer and activator of transcription, ISGF-3: IFN-stimulated gene factor 3, ISRE: IFN-stimulated response elements.

intracellular pathogens such as *Listeria monocytogenes* (Archer, Durack and Portnoy 2014) and *M. tuberculosis* (Manzanillo *et al.* 2012) reduce host defense to infection. In a cutaneous *S. aureus* infection model, induction of STING antagonized innate immunity and allowed infection spread by impairing neutrophil recruitment and IL-1$\beta$ secretion (Scumpia *et al.* 2017).

Bacterial infection-mediated activation of type I IFNs, which influences the regulation of immune and tissue homeostasis, may have detrimental or beneficial consequences for the host (Kovarik *et al.* 2016). *Staphylococcus aureus* invades both phagocytic and non-phagocytic cells (Kintarak *et al.* 2004), and induces type I IFN signaling in various cell types (Kasahara *et al.* 1982; Smith, Johnson and Blalock 1983; Svensson *et al.* 1996; Liljeroos *et al.* 2008; Parcina *et al.* 2008; Martin *et al.* 2009; Kaplan *et al.* 2012; Parker and Prince 2012). Depending on context and site of infection, *S. aureus*-induced type I IFN signaling has been found to be either adverse (Martin *et al.* 2009) or protective (Roquilly *et al.* 2010; Kaplan *et al.* 2012; Lizak and Yarovinsky 2012) to the experimentally infected host. For instance, in models of primary respiratory infection with *S. aureus* using *Ifnar*$^{-/-}$ mice, type I IFN signaling appears to play a negative role in the outcome of infection (Martin *et al.* 2009). In contrast, in a subcutaneous model of infection, the induction of *Ifnb* expression is suggested to be important in controlling the infection. Indeed, supplemen-

tation of IFN-$\beta$ to mice in a skin model of infection resulted in enhanced clearance of *S. aureus* and reduced lesion sizes (Kaplan *et al.* 2012). Pathogenicity of *S. aureus* strains has been mainly correlated to the expression level of type I IFNs induced during infection and resulting exaggeration of inflammatory responses (Parker *et al.* 2014). However, the full role of type I IFNs on the outcome and pathogenesis of staphylococcal infection and involvement of PRRs remains poorly understood. Future studies may open novel avenues in immunomodulatory therapy.

## Collaboration between PRRs during *S. aureus* infections

PRRs as mediators of innate immunity orchestrate together to ensure host protection from pathogen invasion by fortifying epithelial barriers and cellular defenses. Collaboration and interplay broaden the spectrum of activity of PRRs, enabling integration of various pathways. However, the molecules that are associated with the synergistic activation of the several pathways merits further investigation.

MBL, as described above, functions as an opsonin and is involved in activation of the lectin pathway of the complement system through recognition of polysaccharide (i.e. glycan) (reviewed in van Kooyk and Rabinovich (2008); Ip *et al.* (2009)). Accumulating studies suggest a collaboration between MBL and

TLRs (Wang *et al.* 2011; Liu *et al.* 2014), which is demonstrated to modulate cytokine responses during bacterial and viral infections (Jack *et al.* 2001; Ip *et al.* 2008; Ling *et al.* 2012). For example, MBL cooperation with TLR2/TLR6 is initiated upon engulfment of *S. aureus* into the phagosome of macrophages. For this purpose, MBL binds to the LTA and complexes with TLR2 to enhance ligand delivery, resulting in enhancement of NF-κB activation (Ip *et al.* 2008).

The synergistic interplay between TLR2 and NOD2 (Kobayashi *et al.* 2005; Chen *et al.* 2008; Volz *et al.* 2010; Schaffler *et al.* 2014) contributes to both induction or inhibition of specific immune responses (Netea *et al.* 2004). Dendritic cells (DCs) are one of most important innate immune cells. The interplay between NOD2 and TLR2 results in cellular activation observed in murine DCs stimulated with *S. aureus*-derived PAMPs (Volz *et al.* 2010). TLR2 can boost the NOD2 response to *S. aureus*, and NOD2-TLR2 co-stimulation leads to increased activation and maturation of DCs (Schaffler *et al.* 2014). Notably, DCs lacking TLR2 were unresponsive to *S. aureus*-derived peptidoglycan (Volz *et al.* 2010).

In addition, the cumulative effect of NOD2 and TLR2 activation in murine keratinocytes from oral epithelium resulted in induction of IL-6 and IL-1 β secretion. Importantly, the production of these cytokines was reduced by 50% in the absence of either NOD2 or TLR2 (Muller-Anstett *et al.* 2010). However, NOD2 is shown to downregulate TLR1/2 mediated IL-1 β gene expression in macrophages (Dahiya, Pandey and Sodhi 2011). In line with that, NOD2 stimulation, but not NOD2-TLR2 costimulation, promoted intestinal homeostasis through downregulation of inflammatory pathways. This is supported by the finding that activation of NOD2 protected mice from experimental staphylococcal colitis (Watanabe *et al.* 2008). Furthermore, a loss of function due to mutations in NOD2 increases the inflammatory pathogenesis of Crohn's disease (Schaffler *et al.* 2014).

A selective form of autophagy, xenophagy, is involved in the degradation of long-lived cellular proteins, organelles or microbes within eukaryotic cells. In this process, targets are recognized, whereupon entrapment and sequestration of infectious agents into autophagic vesicles is promoted. Thereafter, lysosomes fuse with these autophagic vesicles to degrade their contents. Xenophagy is important for the direct and indirect killing of intracellular and extracellular pathogens, for the generation of bactericidal peptides, as well as for antigen presentation (Sorbara and Girardin 2015). Autophagy can also serve to limit the availability of inflammasome components and decrease proinflammatory signaling (Levine, Mizushima and Virgin 2011; Shi *et al.* 2012). In host cells, both TLR- and NLR-stimulation is associated with induction of xenophagy as a method of defense against bacterial infection (Netea-Maier *et al.* 2016). While xenophagy enhances host cell tolerance to *S. aureus* toxins (Maurer, Torres and Cadwell 2015), defective xenophagy is associated with cystic fibrosis and leads to overproduction of inflammatory cytokines as well as failure of *S. aureus* clearance (Jarry and Cheung 2006). Impaired xenophagy is also associated with Crohn's disease development in patients with NOD2 mutation, since NOD2 is needed to promote autophagosome formation (Travassos *et al.* 2010; Caruso *et al.* 2014). Thus, restoration of xenophagy may be considered as a promising area for research on novel therapeutic strategies targeting the intracellular stages of *S. aureus* infection.

Both TLR2 and NOD2 are known to participate in autophagy and phagocytosis activation via JNK signaling upon *S. aureus* infection (Fang *et al.* 2014). However, the exact type of autophagy triggered by the bacteria has not been described so far. To evade killing by nonprofessional phagocytes, *S. aureus* can subvert xenophagy by inhibiting autophagosome maturation. By this, staphylococci replicate within the autophagosome, and subsequently escape to the cytoplasm whereupon they can induce host cell death (Schnaith *et al.* 2007; O'Keeffe *et al.* 2015; Soong *et al.* 2015). Furthermore, *S. aureus* can evade keratinocyte-mediated clearance in skin infection. Differentiating keratinocytes, which generally recognize *S. aureus* via NOD2, are actively undergoing autophagy. The bacterium activates caspase-1-mediated bacterial clearance through pyroptosis (Aymard *et al.* 2011). If keratinocytes fail to induce clearance, intracellular staphylococci stimulate autophagy, which in turn promotes degradation of inflammasome components, ultimately aiding the survival of *S. aureus* (Soong *et al.* 2015).

All these findings reflect the synergy between various PRRs, their signaling cascades and induced defense mechanisms against *S. aureus,* and also how the pathogen uses multiple vantage points to circumvent these defenses. Moreover, this collaborative activity could potentially be exploited to modulate or improve the host response against pathogenic bacteria that induce an exacerbated inflammatory response.

## CONCLUSION REMARKS AND FUTURE PERSPECTIVE

A fundamental role of the innate immunity is to evoke an appropriate response against invading pathogens or to tissue injury. However, genetic polymorphisms and consequently the expression levels of receptors for bacterial virulence factors can determine the mode and manner of host immune response (Peacock *et al.* 2003; Johannessen, Sollid and Hanssen 2012; Weidenmaier, Goerke and Wolz 2012). *Staphylococcus aureus*, like many other pathogens, evolved various ways to induce tolerance or evade eradication by the host immune system. A wide range of bacterial proteins has been identified that interfere with the induction of proinflammatory responses (Gordon and Lowy 2008; Miller and Diep 2008; Krishna and Miller 2012). Importantly, these antiinflammatory virulence factors targeting NF-κB and MAKP pathways can be served as templates in the development of therapeutic agents to prevent, dampen, or treat chronic inflammatory conditions caused by dysregulation of the very same signaling pathways. Thus unraveling of the underlying mechanism of staphylococcal survival in host, besides a basic understanding of infection process, promotes two important fields of drug development: discovery of novel target points for antimicrobial therapy and new immune-modifying applications to fight against inflammatory diseases.

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