Role of TLR-/NLR-signaling and the associated cytokines involved in recruitment of neutrophils in murine models of *Staphylococcus aureus* infection

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**Key words:** *S. aureus*, Toll-like receptor, murine models, neutrophils, innate

Recent studies in murine models of *Staphylococcus aureus* (*S. aureus*) infection have investigated the association of Toll-like receptor (TLR)2, TLR4, myeloid differentiation factor 88 (MyD88) and Nod-like receptor 2 (NOD2) with the production of cytokines/chemokines and their involvement in the recruitment of neutrophils, which mediate innate immune responses at infection sites. The availability of gene-knockout mice provided opportunities to analyze the redundant roles for specific recognition receptors in our understanding of the innate immune responses that contribute to staphylococcal disease pathogenesis. Emerging findings reveal that the role of recognition receptors in the innate host immune defense and inflammation elicited during infection is influenced both by the nature of *S. aureus*-associated pathogen-associated molecular patterns (PAMPs) and the site of infection highlighting the complex nature of these in vivo interactions. Different susceptibilities have been observed in the various gene-knockout mice regarding clinically relevant infection models. This review details our current knowledge and indicates that further studies are needed to elucidate the contribution of TLR-/NLR-signaling at different sites of staphylococcal infection.

**Introduction**

*S. aureus* is an important bacterial pathogen causing a diverse spectrum of infections in humans which include many superficial, systemic and nosocomial infections involving various tissues such as skin and soft tissue, bone, joints, lungs and brain. A broad range of virulence factors enable *S. aureus* to cause multiple types of infections which can involve any organ system. The diseases caused by *S. aureus* include toxinoses, skin lesions and wound infections as well as severe systemic infections. This Gram-positive bacterium produces a variety of toxins that are associated with various diseases: e.g., toxic shock syndrome toxin (TSST) and toxic shock syndrome (the exfoliative toxins A and B (ETA; ETB), staphylococcal scalded skin syndrome, impetigo, atopic dermatitis, Panton-Valentine leukocidin (PVL) and skin diseases as well as necrotizing pneumonia and staphylococcal enterotoxins A and B (SEA; SEB) and food poisoning. The invasion of the vascular system by *S. aureus* leads to bacteremia, resulting in endocarditis, metastasis and sepsis. *S. aureus* infection may also lead to the formation of abscesses in many organs and induces serious lung problems in cystic fibrosis patients. However, despite its pathogenic properties, most *S. aureus* strains are able to establish an asymptomatic carrier state and only when the host defenses are compromised, there is higher incidence of staphylococcal diseases. Asymptomatic colonization of various organs such as the human skin or the nasal tract is a special feature of several inflammatory diseases where this pathogen may invade deeper tissues causing severe disease. A clinically important aspect of staphylococcal infections is that among both nosocomial and community-acquired infections there is increasing emergence of strains that are resistant to multiple antibiotics such as methicillin-resistant *S. aureus* (MRSA) and multiple-drug resistant invasive strains which pose treatment problems.

Staphylococcal disease severity is determined by the balance between this organism and the host innate immune defense system. Neutrophils (polymorphonuclear phagocytes, PMNs), one of the body’s most abundant but very short-lived leukocytes, are among the first cells to arrive at the site of most bacterial infections or septic injuries. These cells represent a key component of both microbiocidal and acute inflammatory responses and are replaced by monocytes (which differentiate into tissue macrophages) and T cells during the chronic phase of the disease. It has long been recognized that after incidental invasion, *S. aureus* evokes an acute inflammatory response where host neutrophils are rapidly mobilized to the site of infection. Experimental depletion of neutrophils prior to infection with *S. aureus* has been shown to increase disease severity and mortality in animal models mimicking a brain abscess, pneumonia, sepsis and skin and wound infections. The diverse mechanisms (such as reactive oxygen species (ROS), microbiocidal products stored in granules) utilized by neutrophils to kill various pathogens including *S. aureus* have been recently reviewed in references 14 and 15.

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Submitted: 03/16/11; Revised: 06/20/11; Accepted: 06/21/11  
DOI: 10.4161/viru.2.4.16142
A novel neutrophil-mediated anti-bacterial mechanism against *S. aureus* involves the release of neutrophil extracellular traps (Nets) containing DNA, histone proteins and antimicrobial enzymes.\(^{16}\) NETs are made extracellularly by activated neutrophils and have been shown to disarm and kill *S. aureus* extracellularly (NETosis).\(^{16}\) The authors suggest that granule proteins and chromatin together lead to a extracellular complex which induces antimicrobial effects due to a high local concentration of effector molecules. There composition may very well also serve as a physical barrier, which would prevent the spread of *S. aureus*. Further studies showed that NETs are released in a specialized form of cell death that depend on NADPH oxidase.\(^{17}\) Elaborating on these studies the presence of NETosis in lung tissue of *S. aureus*-infected mice has been shown postulating that they may contribute to host immune defense;\(^{18}\) however, detrimental effects of NETosis have also been shown in a study where NET amounts correlated with impaired obstructive lung function in individuals with cystic fibrosis.\(^{19}\)

Although most phagocytosed organisms are killed by neutrophils, some survive intracellularly and multiply within these cells\(^{20}\) by evolving various strategies to resist or to escape killing by neutrophils and establish infections.\(^{21}\) More recent studies have disclosed that neutrophils also potentially contribute to staphylococcal disease pathogenesis by triggering an intense inflammatory response.\(^{22}\) Accordingly, the termination of infection induced inflammation by regulation of neutrophil turnover is crucial for limiting tissue damage.\(^{15}\) The recruitment of neutrophils from circulation reservoirs to the sites of bacterial infection and the activation of their effector functions requires the induction and secretion of a broad set of inflammatory mediators including pro-inflammatory and immuno-modulating cytokines and chemokines (reviewed in ref. 22).

During bacterial infection, pathogen and pathogen-derived molecules termed pathogen-associated molecular patterns (PAMPs), recognize a variety of innate pathogen recognition receptors (PRRs) expressed by immune cells and also non-immune innate cells.\(^{23,24}\) The PAMP theory proposed by Charles Janeway has advanced our understanding of the current concepts of the innate immune response and its relationship to adaptive immunity.\(^{25}\) Of the various types of PRRs, Toll-like receptors (TLRs), a set of membrane-associated PRRs and non-TLRs, such as intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), represent principal innate immune sensors involved in the detection of a broad range of PAMPs. Ligand binding to PRRs triggers signaling cascades leading to the production of various types of mediators including several pro-inflammatory cytokines and chemokines involved in innate immunity (extensively reviewed in refs. 23–27). Depending on the molecular nature of the pathogen-derived stimulus, the resident target sentinel cell type and the associated PRRs may vary in different host tissues as well as in different host species.\(^{28}\) This results in varying profiles of the secreted cytokines/chemokines and their cognate receptors at the site of infection in different organs, which direct the response pathways of the neutrophils in the peripheral compartments.

The precise cellular and molecular mechanisms involved in the recruitment and activation of neutrophils to the site of various types of staphylococcal infections is a subject of intensive investigations during recent years. The aim of this review is to highlight recent studies in experimental murine models describing the involvement of TLR and NOD2 sensing or signaling and the contributions of various cytokines and chemokines in the recruitment of neutrophils mediating innate immune responses in diverse types of infections elicited by *S. aureus*. In order to facilitate better understanding of these studies, the first part of this article provides a brief description of the general features of host TLR/NLR interactions with PAMPs associated with the production of pro-inflammatory cytokines/chemokines involved in the attraction of neutrophils to the initial site of infection.

**Pathogen Recognition by Pathogen Recognition Receptors**

Recognition of invading pathogens is based on germ-line encoded receptors termed PRRs which are expressed by various resident sentinel cells of the innate immune system. PRRs detect conserved microbial molecules/structures, designated PAMPs, which are found in pathogens but are absent from or masked in the host cells.\(^{23}\) This recognition event of the innate immune system allows a rapid response to invading pathogens which includes chemokine production and leukocyte recruitment.\(^{23,24}\) PRRs can recognize host structures or self molecules which are produced during infection, inflammation or tissue damage and are termed danger- or damage-associated molecular patterns (DAMPs).\(^{29,30}\) Based on their localization and functions, PRRs have been classified into three families:

1. **endocytic receptors** such as the glycosylphosphatidylinositol-anchored lipopolysaccharide receptor CD14, scavenger receptors (SR) and C-type lectin receptors (CLR), all of which are involved in the recognition and internalization of microbes and their products

2. **bridging PRRs** such as collectins, ficolins and pentraxins that are soluble PRRs secreted into the extracellular space and that facilitate the recognition (opsonization) and elimination of their ligands by phagocytes and are involved in the activation of complement and

3. **PRRs such as TLRs and NLRs** that are involved in the activation of inflammatory signaling and that represent a crucial component of both the innate and adaptive host defense system.\(^{25}\) Members of the TLR family sense the presence of PAMPs either at the cell surface or in the endosomes, whereas the cytosolic NLR recognizes PAMPs only in intracellular compartments.

**Toll-Like Receptors**

TLRs represent one family of PRRs by which the innate immune system senses the PAMPs of a broad range of pathogens as well as endogenous danger molecules released from host cells as a result of infection or inflammation.\(^{23,33}\) TLRs are transmembrane proteins expressed on various immune and non-immune cells such as B cells, NK cells, dendritic cells, macrophages, mast cells, fibroblasts, epithelial cells and endothelial cells. To date, at least 13 different TLRs (10 in humans and 13 in mice) have
been identified. TLRs are located either at the cell surface like TLR1/2, -2/6, -4 and -5 or within endosomal compartments such as TLR3, TLR7, TLR8 and TLR9. Each TLR is capable of sensing different PAMP subsets and activating distinct cellular responses. TLR2 recognizes specific components such as lipoproteins or lipopeptides. TLR1 and TLR6 complex with TLR2 and discriminate between triacyl- and diacyl-lipopeptides, respectively. TLR4 recognizes endotoxin or LPS, TLR5 recognizes bacterial flagellin, TLR3 and TLR7 and TLR9 sense bacterial-, viral- or host-derived nucleic acids, and TLR11 (found only in mice) is required for the response to uropathogenic bacteria and profilin-like protein from Toxoplasma gondii.

All TLRs have an extracellular sensing leucine-rich repeat (LRR) domain, a transmembrane domain, and a highly conserved cytoplasmic Toll- and interleukin-1 (IL-1) receptor (TIR) domain involved in mediating intracellular signaling pathways. The variations in the extracellular domains of TLRs determine the ligand binding specificity of different TLRs.

Following ligand binding, downstream intracellular signaling is initiated via homophilic TIR-TIR interactions with the TIR-domain containing adaptor proteins belonging to the IL-1R family. TLRs recruit different combinations of four adaptor proteins: myeloid differentiation factor 88 (MyD88), TIR-domain-containing adaptor protein (TRAP), also known as myeloid adaptor-like protein (MAL), TIR-domain-containing adaptor inducing IFNβ (TRIF), and TRIF-related adaptor molecules (TRAM). The role of the fifth adaptor protein, SARM (sterile-α and HEAT/Armadillo motif-containing protein) is to negatively regulate TRIF-dependent TLR-signaling. MyD88 is used as an adaptor by all TLRs except TLR3, which exclusively uses TRIF as an adaptor. TLR2 and TLR4 both use MyD88 and MAL as a bridge adaptor for the coupling of MyD88 to TLR. MyD88 is directly activated by other TLRs in the absence of MAL and TRIF is used by TLR3 and TLR4 and activates a MyD88-independent signaling pathway. TLR 4 is the only TLR that engages both MyD88-dependent and TRIF-dependent signal transduction pathways. TLR4 requires the assistance of another bridging adaptor TRAM for recruitment of TRIF for the MyD88-independent pathway. In addition to the direct role of MyD88 in IL-1R-, IL-18R- and TLR-signaling, MyD88 functions in other signaling pathways such as IFNβ signaling.

TLR signaling leads to the activation of transcription factors, such as nuclear factor kappaB (NFkB) and activator protein 1 (AP1), which are common to all TLRs, resulting in the production of inflammatory cytokines and chemokines. Some TLRs can also activate interferon-regulatory factors (IRFs), leading to the production of type 1 interferons (IFNs) such as IFNα family and IFNβ and chemokine RANTES (Regulated on Activation Normal T cell Expressed and Secreted). TLRs activating mitogen-activated protein kinase (MAPK)-regulated transcription factors such as p38 and c-Jun-N-terminal kinase (JNK) induce the expression of genes coding for a variety of proteins including several inflammatory cytokines. Subsequently, the cytokines and chemokines initiate and amplify inflammatory responses by recruiting and activating appropriate cells such as neutrophils. Although different TLRs detect different pathogen components, they upregulate many of the same genes which include the inflammatory chemotactic cytokine cluster.

NOD-Like Receptors

NLRs represent cytosolic proteins that recognize PAMPs in the intracellular compartments. Two key functions of NLRs are: (1) mediating the activation of the intracellular signaling cascade leading to the activation of NFkB and (2) the production of cytoplasmic multiprotein complexes known as inflammasomes, which mediate the activation of inflammatory caspases leading to the production of inflammatory cytokines (reviewed in refs. 27 and 44). Caspases also mediate cell death after infection. Similar to TLRs, NLRs contain a C-terminal domain consisting of tandem repeats of LRR, which are involved in ligand sensing and a centrally located nucleotide binding NOD domain which is required for self-oligomerization and for the activation and recruitment of downstream signaling proteins. The variable N-terminal domains can contain a caspase recruitment and activation domain (CARD), a pyrin domain (PYD), baculovirus inhibitor of apoptosis protein repeat (BIR) domain or a so-called death effector domain (DED).

Over 23 NLRs have been identified in humans and mice, but the function and specific ligands have been identified only for a few of them. NLRs include proteins such as NOD1 (also known as the caspase recruitment domain 4, CARD 4), NOD2 (also known as CARD 15), NALPs [NACHT domain-, leucine rich repeat- and pyrin-domain-containing proteins (NALP1 and NALP3—also known as cryopyrin-) and interleukin (IL-1β)-converting-enzyme-protease-activating factor (IPAF). NOD1 and NOD2 are the two best characterized NLRs that have so far shown to induce intracellular signaling following their recognition by bacterial components. Ligation of either receptor with appropriate ligands activates several signaling cascades including MAPK- and NFkB-pathways leading to the production of pro-inflammatory cytokines and chemokines that recruit immune cells to the sites of microbial infection. As is the case with TLR ligands, NOD agonists are shed and recycled during bacterial growth and infection. NOD1 detects meso-diaminopimelic acid, which is a part of Gram-negative peptidoglycan and the ligand for NOD2 is the muramyl dipeptide (MDP) motif of peptidoglycan found in both Gram-negative and Gram-positive bacteria. Although there is ample in vitro evidence showing that NOD1 and NOD2 mediate bacterial sensing, in vivo observations of their role in host defense are limited to models of gastrointestinal tract infections. There is increasing evidence that NOD1 and NOD2 cooperate with TLRs to produce pro-inflammatory cytokines and antibacterial molecules and shape the host response to bacteria.

Other NLRs such as the NACHT domain-leucine rich repeat- and pyrin-domain-containing (PYD)-containing protein (NALP 1 and NALP3) and interleukin (IL-1β)-converting-enzyme-protease-activating factor (IPAF) are involved in the formation and activation of large, multimeric protein complexes named inflammasomes, which control the activation of caspase-1, and the processing and secretion of caspase-1-dependent cytokines.
Caspase-1 is the first discovered member of a family of conserved proteases which cleave a variety of cellular substrates and are involved in a number of essential functions including inflammation. Multiple inflammasomes have been identified and defined by the NLR protein they contain.55 So far the ability to activate caspase-1 has been shown for two NLRs, i.e., cryopyrin and protease-activating factor (IPAF).36 Cryopyrin is activated by bacterial RNA, toxins and IPAF detects bacterial flagellin.55 For detailed information on TLRs and NLRs refer to available review articles.24,27,45

Cytokines

Cytokines represent a heterogeneous group of secreted regulatory proteins which perform many effector functions including coordination of inflammation. These proteins are produced in all tissues and by a wide variety of cells and mostly represent an emergency response to infection, tissue damage or other insults. Several cytokines have overlapping functions and can compensate each other's functions and also act in synergy to modulate target cell functions. Cytokines are grouped into families based on their structural and/or functional similarities.

Cytokines involved in the inflammatory response can be divided into three groups: (1) pro-inflammatory cytokines which initiate and enhance the cascade of events, (2) IL-6 type cytokines which induce systemic actions and (3) anti-inflammatory cytokines which downregulate the inflammatory response. Based on their functions, cytokines can be divided into pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines, such as IL-1 and TNFα, are produced soon after infection, and are responsible for the acute inflammatory reaction. Anti-inflammatory cytokines, such as IL-10 and TGFβ, downregulate the inflammatory response and enhance the generation of regulatory T cells.58,59 Downregulation of the inflammatory response is important to avoid excess damage to the host and restore homeostasis after inflammation. Inflammatory cytokines (chemokines) are produced locally in infected tissues, where they form chemokine gradients by binding to the extracellular matrix. The gradient is recognized by leukocytes via their cell surface chemokine-receptors and this guides the recruitment of cells to the site of inflammation during infection. Cytokines that enter the circulation include TNFα and IL-6 and they have systemic functions. Although during the early response, cytokines such as TNFα and IL-1β are involved in host defense by recruitment of host neutrophils to the site of infection, their entry into the systemic circulation can lead to microvascular injury. Several cytokines are known for their overlapping functions and can compensate each other's functions and also act in synergy to modulate target cell functions.

TNFα is a multifunctional pro-inflammatory cytokine which can bind to two different types of receptors. TNF-receptor 1 (TNFR1) binds preferentially to soluble forms of TNFα whereas TNFRE2 is preferentially activated by cell-associated TNFα. Binding of TNFα to its cell surface receptor activates MAPK and NFκB transcription factors, which regulate the gene expression of several PRRs, transcription factors, cytokines and anti-microbial mediators. Additionally, the intracellular death domain of the TNFα-receptor may trigger caspase activation and apoptosis upon TNFα-binding.60 Numerous exogenously and endogenously produced leukocyte chemokine attractants that initiate leukocyte-migration and -activation have been identified. These include classical chemo attractants, like formylated peptides, C5a, C3a as well as the superfamily of chemokines that selectively induce infiltration, trafficking and homing of leukocytes. The effects of all of these chemo attractants on their target cells are mediated through specific cell surface receptors, which belong to the superfamily of seven-transmembrane-spanning-G-protein-coupled-receptors (GPCRs).61 PMNs express several members of this receptor family on their surface, including the chemokine receptors CXCR1 and CXCR2 which recognize IL-8 and NAP, GRO or ENA respectively.

Chemokines have been classified into four groups on the basis of conserved N-terminal cystine residues (CC, CXC, CX3C and C families). The CC family chemokines are chemo attractants to one or several types of mononuclear leukocytes, eosinophils or basophils.62,63 CXC chemokines are classified on the basis of the presence or absence of an ELR (glutamine acid, leucine-arginine) motif. ELR-positive (ELR+) CXC chemokines attract and activate neutrophils and ELR-negative (ELR-) CXC chemokines recruit T lymphocytes.64-66 Chemokines can be divided on the basis of their functionality into homeostatic and inflammatory chemokines. Inflammatory chemokines include several members of the CC and CXC chemokines and they mainly attract monocytes, neutrophils, NK cells, immature DCs and T cells. During early stages of infection, activated macrophages release cytokines such as TNFα, IL-16, IFNs and a multitude of CC and CXC chemokines, which stimulate precursor cells from the bone marrow, resulting in elevated levels of neutrophils.

The expression of cytokines and chemokines genes is controlled primarily at the transcriptional level by binding of transcription factors to their target elements on gene promoters. Most of these genes are regulated by NFκB, IRF, STAT (signal transducers and activators of transcription) and MAPK-activated transcription factors. These are also the transcription factor pathways most prominently activated via PRRs. The induction of pro-inflammatory cytokines by TLR ligands is predominantly mediated by MyD88 signaling pathways. The TLRs share an intracellular signal with the two cytokine receptors IL-1R and IL-18 (formerly designated IFN-inducing factor and is produced by macrophages). The receptor signal is activated via MyD88, IL-1R-associated kinase (IRAK-1), TNFR-associated factor and TGFβ-activated kinase leading to nuclear translocation of NFκB.43

However, cytokines themselves also activate these transcription factors, leading to positive and negative feedback mechanisms. Thus, both direct PRR-mediated and indirect (cytokine-mediated) mechanisms are involved in the regulation of cytokine and chemokine production during microbial infections.58 Cytokines mediate their functions via specific cell surface receptors that initiate intracellular signaling pathways and gene expression.58 CXC chemokines act via CXCRs expressed on the surface of myeloid and non-myeloid (resident) cells. Five CXCRs have been identified (CXCR 1 to 5). CXCR1 and CXCR2 are
the receptors of ELR+ CXC chemokines, whereas CXCR3 is the receptor for ELR CXC chemokines. All known murine neutrophil chemotactic proteins including KC (Keratinocyte-derived chemokine, which is the functional murine homolog of IL-8), MIP-2 (Macrophage inflammatory protein), CXCL5 and lung-kine bind to murine CXCR2.

S. aureus-Associated Pathogen-Associated Molecular Patterns

S. aureus expresses a wide variety of antigens which can potentially present many different PAMPs as targets for recognition by the host’s innate immune system. A review of various in vitro studies reveals a large number of reports showing that S. aureus and its isolated cell wall components such as peptidoglycan, lipoteichoic acid (LTA) and lipoproteins serve as ligands recognized by host TLR2. The cell wall of S. aureus is composed of multiple layers of peptidoglycan and due to its repeat chain of alternating N-acetylglucosamine and N-acytethylmuramic acid residues, it is an ideal ligand for TLR2-recognition. Peptidoglycan and LTA are also capable of recognizing mannose-binding lectin and ficolins on host neutrophils and macrophages. This recognition results in the activation of the lectin pathway whereby the complement subunit C3b binds to the cell surface of S. aureus and is subsequently recognized by the complement receptor (CR) 1 on phagocytes. Peptidoglycan can also directly activate the alternate complement pathway. C5a formation also recruits additional neutrophils and macrophages to the site of infection. It has been shown that peptidoglycan-embedded molecules, which bind to TLR2, downregulate S. aureus superantigen-induced T cell activation and thus prevent toxic shock syndrome. Apparently, this is achieved via induction of interleukin 10 production by inhibiting the IL-2 response to staphylococcal superantigens.

More recent studies have identified Panton-Valentine leukocidin, α-toxin and protein A as virulence factors of S. aureus pneumonia. Protein A, which is a cell wall-associated protein, binds directly to tumor necrosis factor receptor-1 (TNFR-1) on respiratory epithelium and this TNF-signaling activates a chemokine-/cytokine-signaling cascade. Reported, protein A sensitizes B cells for the recognition of TLR2-binding lipopeptides. Stimulation with both TLR2-binding molecules and lipopeptides increases the expression of IgM antibodies within the B cells. α-toxin, which is responsible for pore formation and cell wall lysis in host erythrocytes and monocytes is also involved in significant upregulation of cytokines such as IL-1β, IL-6 and TNF. α-toxin exposure has been shown to be a strong neutrophil attractant to the site of infection. Bacterial genomic CpG-DNA represents a PAMP which can activate innate immune cells such as macrophages and DCs to secrete cytokines. The observation that S. aureus DNA induces production of cytokines such as TNFα, IL-12 and IFNγ and can elicit an inflammatory response in a cutaneous mouse model raises questions about a possible involvement of TLR9 in S. aureus infections. S. aureus also secretes N-formyl peptides at the infection site that bind directly to the formyl peptide-receptor expressed on host leukocytes and are responsible for recruiting host neutrophils and macrophages. PRRs involved in the detection of S. aureus also include NOD2, which recognizes peptidoglycan fragments.

S. aureus Infections in Murine Models

Toll-like receptor signaling, cytokines and neutrophil recruitment. Innate receptors such as TLRs are expressed by epithelial barriers that allow them to discriminate pathogens and toxins from innocuous moieties and thereby protecting various tissues and organs against infections. There is increasing evidence implicating that different epithelia have developed distinct mechanisms involving signaling of innate receptors via PAMPs to protect different anatomical sites against pathogens and that these may be site-specific. Thus, for a precise understanding of S. aureus interactions with TLRs in vivo (in a setting where multiple receptors and multiple cell types exist), it is necessary to consider the interactions in connection with various target tissues associated with S. aureus infections (Table 1).

| Site of S. aureus Infection | Toll-like receptor 2 |
|-----------------------------|---------------------|
| Blood                      | 89                  |
| Abdominal                  | 90                  |
| Skin                       | 91                  |
| Lung                       | 92                  |
| Brain                      | 93                  |
| Cornea                     | 94                  |

TLR2 is involved in the recognition of a variety of pathogens and their components including Gram-negative and Gram-positive bacteria such as S. aureus. The ability of TLR2 to recognize a broad range of different ligands is partially explained by its association with either TLR1 or TLR6. Additionally, TLR2 can recognize zymosan in association with the structurally unrelated C-type lectin family known as dectin-1. Also, both CD14 (monocyte differentiation antigen which is a glycosylphosphatidylinositol-linked receptor) and CD36 (scavenger receptor) are involved in TLR2-mediated recognition as co-activating molecules. TLR2 triggers cytokine release via the activation of the NFkB pathway. TLR2-signals are transported to cytoplasmic NFκB via adaptors such as MyD88, TIRAP/MAL and several types of kinases. Cytoplasmic NFκB binds to DNA as a dimer and is prevented from entering the nucleus due to the blocking effect of a bound IκB molecule. Phosphorylation of IκB by IKK kinases results in the dissociation of IκB from NFκB and NFκB is translocated into the nucleus leading to the transcription of immune response genes.

Over the past several years, several studies in vitro have demonstrated the contribution of several isolated PAMPs from S. aureus in the activation of TLR2-signaling cascades leading to the production of inflammatory mediators including cytokines and chemokines. More recently, the involvement of TLR2 has been implicated in the host innate immune response in a number of murine models of staphylococcal infection, which are reviewed below.
Septicemia/Sepsis

Sepsis is the result of a complex cascade of events resulting in multi-organ failure that include abnormal cytokine activation, neutropenia and coagulation dysfunction.45 Reportedly, both TLR2- and MyD88-knockout mice but not wild-type (WT) mice succumb to intravenous infection with *S. aureus* with more enhanced susceptibility in MyD88-knockout mice.49 In this study, increased susceptibility of knockouts (KO) was observed with high-dose but not low-dose infection and susceptibility of knockouts also correlated with significantly higher bacterial loads in the kidneys and in blood.89 It has been demonstrated that *S. aureus* lipoproteins contribute to the pathogenesis of *S. aureus* sepsis in intravenously infected C57BL/6 mice via TLR2, with IL-1β chemokine-mediated inflammation and high bacterial numbers.70 In this study, TLR2-KO mice allowed inflammation and limited bacterial growth only in the kidneys but not in the spleen and liver and also showed improved recovery.70 Lack of MyD88 allowed a much stronger growth in the kidneys and knees, suggesting that MyD88-induced effects protect the host’s kidneys, but not its liver. The authors proposed an organ-specific TLR2-mediated response, but an overall moderate role of TLR2 signaling in *S. aureus* sepsis as published earlier for *S. aureus* skin infections.91

C57BL/6 mice infected intravenously with *S. aureus* show significant resistance in terms of bacterial growth and survival when compared to other mouse strains such as A/J mice.89 It was concluded that the superior genetic resistance of C57BL/6 mice was due to rapid peak level expression of the chemokine CXCL1 in the kidneys by the resident cells (only 1 h after infection) that induced neutrophil recruitment resulting in the control of infection.89 *S. aureus* variants lacking lipoprotein precursors fail to elicit an adequate immune response (minimal neutrophil infiltration) and this results in lethal infections with disseminated abscess formation in intravenously infected animals.92 The recognition of lipoproteins is mediated via TLR2 in an NFκB-dependent fashion which induces the production of inflammatory cytokines.29

In a *S. aureus*-induced septic mouse model, cardiac dysfunction was associated with increased myocardial TNFα- and IL-1β-synthesis and was observed only in TLR2-expressing WT, but not in TLR2-deficient mice.90 Thus, in this model deficiency of TLR2 exerted a protective effect associated with decreased levels of TNFα and IL-1β. Cardiac performance of isolated rat hearts perfused with purified staphylococcal LTA has been shown to be depressed via activation of myocardial TNFα synthesis.91 The importance of IL-1R-signaling in *S. aureus* infection for the controlling of bacterial growth and for the subsequent protection against septic death and septic arthritis was demonstrated using IL-1R-deficient mice which showed increased susceptibility as compared to WT mice following intravenous infection with *S. aureus*.92 The data presented underscored the importance of IL-1R-signaling for controlling production of pro-inflammatory cytokines (TNF, IL-6, IL-1β and IL-18) in response to *S. aureus* and revealed fewer neutrophils in the blood of infected IL-1R-deficient mice.92 The interleukin 1-receptor-associated kinase (IRAK-1) is known to transduce IL-1R and IL-18R signals involved in cytokine production and neutrophil recruitment.93 Evidence obtained in IRAK-1-deficient mice revealed that at a high dose of *S. aureus* (2 x 10^7^ bacteria), these mice show increased susceptibility compared to WT controls, whereas unlike IL-1R-deficient mice, they remain resistant to a low dose of *S. aureus* (10^3^). The underlying mechanism(s) by which IRAK-1 mediates protection (dependency of IL-1R, IL-18R or TLR) remain(s) unclear.

The studies detailed above, demonstrate that TLR2 requirement in *S. aureus*-induced septic models should be discussed in an organ specific context. Whereas TLR2 ablation has the negative effect of increasing the bacterial load in the kidneys, its deficiency correlates with a protective cardiac effect. Furthermore the role of TLR2 in sepsis is probably moderate as both MyD88 and IL-1R deficiency show a more pronounced effect.

Peritonitis

Mice challenged with peptidoglycan (PGN) or live *S. aureus* showed a significant accumulation of neutrophils during the early stage (4 h) of infection, which was partially TLR2-dependent and also involved C5aR. However, by 24 h, the response was independent of TLR2 and C5aR. Bacterial clearance from the spleen and peritoneum and survival was not altered in TLR-KO mice versus WT mice.90 These observations implied that lack of TLR2 was inconsequential with respect to *S. aureus* clearance and that neutrophil recruitment was partially TLR2-dependent. *S. aureus* inoculated intraperitoneally in mice deficient in a gene that regulates neutrophil migration (IAP) resulted in less neutrophil accumulation, better bacterial clearance and less mortality compared to infection of WT mice.90 In addition, mice with a partial depletion of neutrophils prior to infection revealed better bacterial clearance, diminished neutrophil accumulation and less mortality compared to infection of mice which were completely depleted of neutrophils or WT controls.90 As well, local release of CXC chemokines promoted PMN migration and created a situation in which PMNs internalized, but did not kill *S. aureus*. Since exogenous administration of CXCL1 and CXCL2 increased mortality and bacterial burden in this model, the authors concluded that CXC chemokines increase the persistence of live *S. aureus* within PMNs and provide the bacteria a niche where they are protected from competent phagocytes.20

Based on these studies no substantial role for TLR2 in peritonitis regarding survival can be observed. Striking however is the postulated detrimental role of neutrophils in this clinical setting, where a decrease in neutrophil accumulation has a strong effect on disease progression. It seems likely that neutrophils can in some clinical settings provide a protective niche for *S. aureus*.

Skin and Wound Infections

In a cutaneous mouse model of *S. aureus* infection,91 TLR2-deficient mice showed only moderately larger lesions and minimal increases in bacterial counts compared to WT controls. On the other hand, both MyD88- and interleukine-1 receptor (IL-1R)-deficient mice showed increased susceptibility to
cutaneous infection with *S. aureus*, with reduced neutrophil infiltration, higher bacterial counts and failure to eliminate the infection (Fig. 1). Additionally, IL-1R- but not TLR2-deficient mice revealed larger lesions and showed defective induction of neutrophil chemokines KC and MIP-2. The authors demonstrated that the resident skin cells (and not the bone-marrow-derived leukocytes) represented the likely source of these chemokines. Similarly, in another type of cutaneous infection model, PMN-depleted mice (using an anti-Gr-1 Mab clone RB6-8C5) developed larger non-healing skin lesions and failed to clear *S. aureus* from these lesions. Thus, IL-1R-deficient mice and neutrophil-depleted mice share a similar phenotype, implying that IL-1R-mediated neutrophil recruitment is crucial for host defense against *S. aureus* skin infection. IL-1α and IL-1β are the known primary ligands that activate IL-1R signaling and it has been demonstrated that following subcutaneous infection with *S. aureus*, C57BL/6J mice deficient in IL-1β, IL-1α/IL-1β, but not in IL-1α, developed larger lesions with higher bacterial counts and had decreased PMN recruitment compared to WT mice. It is known that both TLR2 and IL-1R signal via the

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**Figure 1.** A schematic representation of the major events in the induction of MyD88-dependent TLR2 or IL-1R signaling pathways during skin infection with *S. aureus*. Data obtained from references 91, 104 and 105. Triggering of Toll-like receptor (TLR2) by *S. aureus* is associated with the recruitment of the intracellular adaptor protein, Myeloid Differentiation Factor 88 (MyD88) and leads to the activation of nuclear factor κB (NFκB)/inhibitor of NFκB (IκB) activation in resident innate immune cells of the skin (Keratinocytes/Langerhans cells/Dendritic cells/γ-δ T cells). Following degradation of IκB, NFκB is freed and translocated to the nucleus and induces appropriate expression of genes which are responsible for the transcriptional initiation of a number of cytokines and chemokines such as those belonging to the IL-1 family. It also drives the synthesis of pro-IL-1β and the activation of inflammasome components (caspase-1 and NALP-3) which are involved in the secretion of mature IL-1β. The binding of IL-1β to its receptor (IL-1R) activates many of the same signaling cascades as involved in TLR activation. This signaling pathway stimulates the production of MIP-2 and KC which then promote neutrophil recruitment into the focus of infection thereby creating an inflammatory state and clearance of infection. Activation of the IL-1β/IL-1R pathway in γ-δ T cells leads to the production of IL-17 (besides other cytokines), which binds to its receptor IL-17R expressed on a variety of cell types and triggers the release of inflammatory cytokines that also participate in the mediation of inflammation. NOD2, Nod-like receptor2; NALP3, NACHT domain-, leucine rich repeat- and pyrin-domain-containing protein3; KC, keratinocyte derived chemokine; MIP-2, macrophage inflammatory protein.
adaptor molecule MyD88 and it is suggested that following cutaneous infection downstream signaling events diverge and that TLR2 signals are of redundant nature while those of IL-1R are non-redundant. A more recent study has demonstrated that IL-17 (IL-17 belongs to a recently discovered proinflammatory cytokine family and is an important regulator of inflammation at the interface between innate and adaptive immunity influencing both innate and adaptive immune cells) is required for defense against S. aureus cutaneous infection. The authors showed that IL-17 required IL-1R/MyD88 signaling, TLR2/MyD88 signaling and IL-23 and that resident γ/δT (but not α/β) skin T cells are needed for its production. It was concluded that IL-1 (along with TLR2 and IL-23) promotes neutrophil recruitment to the site of infection by IL-17 production by resident γ/δ T cells. Available evidence demonstrates that the inflammasome (facilitates caspase-1-activation) is required for the generation of active IL-1β by bone marrow (BM)-derived cells that subsequently activate IL-1R-signaling by non-BM-derived resident skin cells for PMN recruitment in the innate immune defense against S. aureus. Earlier, a requirement for the inflammasome component apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) for the generation of active IL-1β has been demonstrated in vitro by using S. aureus stimulated bone marrow-derived macrophages.

CXC chemokine-induced internalization of S. aureus by PMN at the infection site using a murine model of surgical wound infection in which increased IFNγ was produced locally by S. aureus capsule polysaccharide-8 activated T cells has been demonstrated. IFNγ−/− mice demonstrated decreased PMN-specific chemokine production and associated PMN recruitment to the infection site compared to wild-type mice. The reduced PMN influx was associated with a lower tissue bacterial burden, suggesting that a PMN-rich environment potentiates pathogenesis due to the bacterial ability to survive within the PMNs. Although CXCL1 and CXCL2 have been found to be important in neutrophil recruitment in S. aureus wound infection, exogenous administration of CXCL2 resulted in less effective clearance of the bacteria from the wound. Conversely, a blockade of CXCRII, which binds the murine chemokines MIP 2 (CXCL2) and KC (CXCL1) in vivo, significantly reduced the bacterial burden at the infection site. The production of these chemokines has been attributed in part to IL-1β, IL-1R and γ interferon which were upregulated in these studies and the migration of neutrophils could be modulated by CD4+ T cells resulting in less inflammation and more efficient bacterial clearance.

Interestingly, in a non-obese diabetic (type 1 diabetes) mouse model, mice injected with S. aureus in the hind pad, showed exacerbated infection compared to non-diabetic controls. The diabetic mice developed delayed tissue inflammation during early stages of infection (6–12 h), and presented significantly reduced tissue levels of the chemokines KC and MIP-2, which reportedly play an important role in recruitment of PMNs to the site of infection. Likewise, db/db mice with type 2 diabetes proved more susceptible to hind pad infection with S. aureus than age-matched +/+ littersmates but these mice showed elevated tissue levels of pro-inflammatory chemokines which recruit PMNs with greater inflammatory response and early PMN influx than observed with wild-type animals. Apparently, the increased inflammatory response observed in db/db mice did not result in resolution of S. aureus infection, which is in accordance with studies where a neutrophil-rich environment actually exacerbated the infection as a result of S. aureus survival within PMNs. Several studies support the notion that the phagocytosis of bacteria initiates the programmed cell death of PMNs, which are subsequently cleared by infiltrating macrophages. On the other hand, failure of PMNs to undergo apoptosis results in the release of PMN cytotoxic molecules (proteolytic enzymes, antimicrobial proteins and reactive O2 species) as well as pro-inflammatory mediators. This sustained state results in tissue destruction and the progression of an acute inflammation into chronicity or sepsis.

A strong role for MyD88 and the IL-1R in S. aureus clearance is implicated in skin infections. Major events of interest in the MyD88 dependent TLR2 and IL-1R signaling pathways triggered in response to staphylococcal infection in the skin are summarized in Figure 1. As the role of TLR2 seems significantly less important and based on the fact that both TLR2 and IL-1R utilize MyD88 to initiate signaling, the experimental data indicates that differences between TLR2/MyD88 and IL-1R/MyD88 signaling account for differences in susceptibility to S. aureus and are most probably linked to differences in neutrophil recruitment. In this context IL-17 production by resident γ/δ T cells seems to be crucial. For wound infections, the supportive role of neutrophils in skin infections has been challenged and as in some peritonitis models it has been shown that neutrophils can function as a safe haven for S. aureus. Furthermore lack of neutrophil apoptosis could lead to tissue destruction. Therefore skin infections probably reflect a highly diverse and complex interplay between various TLRs and cells which effect clinical progression or clearance of S. aureus.

### Pulmonary Infections

Nasal carriage represents a major source for staphylococcal infection and TLR2-deficient mice infected intranasally showed 10-fold higher nasal carriage of S. aureus when compared to that observed in WT mice. However, MyD88-deficient mice, which are highly susceptible to systemic infection with S. aureus when infected via the intranasal route of infection, have been found to control pulmonary infection with S. aureus and the production of pro-inflammatory cytokines and neutrophil recruitment is unaffected. This observation suggests a TLR2-signaling pathway without the participation of the key molecule MyD88 or alternatively S. aureus is recognized by other TLRs or non-TLR receptor(s) independent of TLR2. Murine models of airborne infection with S. aureus have been useful in characterizing the host response during the first 4–8 h of lung infection. In these studies involving early pneumonia, pro-inflammatory cytokines/chemokines were released and neutrophils were rapidly recruited to the site of infection and cleared the infection within 24–36 h. In a mouse model of S. aureus pneumonia levels of the CXC chemokines (KC and MIP-2) were significantly lower in the airways of mice infected
with a \textit{S. aureus} α toxin-deficient mutant (hla-) than observed in mice infected with hla+ \textit{S. aureus}.

The authors concluded that besides other virulence factors, α toxin is essential for the secretion of newly synthesized CXC chemokines (KC and MIP-2) into the airway for neutrophil recruitment and associated lung injury. These cytokines have also been detected in the bronchoalveolar lavage fluid and pleural fluid after infection with \textit{S. aureus}.

It has been shown that Protein A, which is expressed on \textit{S. aureus} cell walls, interacts with the TNFα-receptor (TNFR-I) on the respiratory epithelium, thereby activating NFκB, and subsequently induces the expression of inflammatory cytokines such as IL-8 leading to the recruitment of neutrophils. Figure 2 shows areas of interest in the signaling pathway of TNFR1 triggered by \textit{S. aureus} virulence factor Protein A in the mouse lung epithelium. Reportedly, mice deficient in TNFR1 that were infected intranasally with \textit{S. aureus} showed less pneumonia, bacteremia and mortality than observed in similarly infected WT mice. Furthermore, absence of Protein A also reduced disease susceptibility and thus it was concluded that Protein A interaction with TNFR1 results in severe infection with pneumonia. Data presented show the shedding of TNFRI from the cell surface into the airway space thereby competing with membrane bound TNFRI for Protein A and TNFα and downregulating the inflammatory response.

Taken together pulmonary infection is distinct in its TLR requirements. Although TLR2-deficiency leads to an increase in \textit{S. aureus} nasal carriage, MyD88-deficient mice can control pulmonary infection with \textit{S. aureus} in contrast to the situation in systemic infection. This could indicate a MyD88-independent TLR2 signaling pathway or the use of other TLR or non-TLR molecules. Indeed taking the unique immunology of the lung into consideration it seems reasonable to suggest that other
cellular receptors, processes and cell types play a role in this organ regarding *S. aureus* clearance.

**Brain Abscesses**

In an experimental mouse brain model, *S. aureus*-induced abscesses are characterized by intense microglial and astrocyte activation along with a strong innate immune response. The severity of brain abscesses is associated with a persistent expression of IL-1β, TNFα and MIP-2/CXCL2.10,93 In the absence of TLR2, the incidence of *S. aureus*-induced brain abscess, the cytokine and chemokine response and the recruitment of inflammatory leukocytes increased significantly as compared to WT mice. WT mice terminated the infection more effectively.93 Besides TLR2-knockout mice, TLR4-knockout mice also showed reduced survival (47% mortality) as compared to 87% mortality among TLR2-knockout mice. The severe clinical disease was associated with high bacterial load (CFU) and elevated levels of pro-inflammatory cytokines (TNF and IFNγ) implying that TLR2 and TLR4 are not required for induction of these cytokines.116 However, in an another *S. aureus* mouse brain abcess model, TLR2-knockout mice showed no adverse effects on survival or bacterial burden.117 It is worth mentioning that TNFα and MIP-2 levels were markedly reduced during early infection stage in TLR2-KO mice. On the other hand, IL-1 and TNFα appeared to be involved in containing bacterial infection in the brain abscesses model, as evident by increased mortality and bacterial burden in IL-1 and TNFα knockout mice compared to WT controls.117,118 Subsequently, these authors presented data showing that whereas innate immune infiltrates were not significantly different between TLR2-KO mice and wild-type mice, but the Th 17 infiltrates and the proinflammatory cytokine IL-17 levels were elevated in the brain abscesses of TLR2-KO mice.119 IL-17 represents the first described member of the recently discovered pro-inflammatory cytokine family and is an important regulator of inflammation at the interface between innate and adaptive immunity influencing both innate and adaptive immune cells. The authors concluded that although TLR2 does not appear to dictate the extent of bacterial replication in brain abscesses, it does influence the pro-inflammatory mediator expression during the acute stage of the disease.

All in all, although the role of TLR2 remains controversial in *S. aureus* induced brain abscesses, it is particularly interesting that as seen in skin infections a pivotal role for TLR2 induced IL-17 expression seems to play a role in pro-inflammatory mediator expression. Therefore even though skin and brain present themselves with distinct differences regarding the clearance of *S. aureus*, IL-17 seems to be a common denominator, illustrating the significance of the TLR2-T17 axis in controlling *S. aureus* infections.

**Corneal Infections**

Compared to WT mice, TLR2-deficient mice show reduced corneal inflammation associated with reduced corneal levels of chemo attractants for neutrophils and monocytes such as KC and MIP-2.94 This results in diminished neutrophil infiltration at the site of infection in knockouts, implying the involvement of TLR2 in the mobilization of neutrophils to the infected corneal epithelium. TLR9-deleted mice failed to impair *S. aureus* infection in the corneal epithelium.94 Whether TLR9 is involved in *S. aureus* infections is still not clear.

The data regarding *S. aureus*-induced corneal infections remains scarce. However, lack of TLR2 does impair neutrophil recruitment. The observation that TLR9 ablation does not effect *S. aureus* infection in the cornea appears to rule out a role for the recognition of CpG motifs in *S. aureus*-induced inflammation.

**NOD-Like Receptor: Signaling, Cytokines and Neutrophil Recruitment**

The NLR-family of proteins such as NOD1 and NOD2 are thought to play a role in controlling the cytokine responses to bacterial antigens. Members of the NOD family have been identified as being responsible for intracellular responsiveness to components of PGN.49 Mice deficient in NOD2 have been used to study the role of this cytosolic sensor in *S. aureus* infection. Intraperitoneal *S. aureus* infection of NOD2-deficient mice resulted in increased susceptibility and significantly higher bacterial load in various organs compared to that observed in TLR2-deficient mice or WT mice.120 Furthermore, although NOD2-deficient mice showed normal neutrophil recruitment, there was defective neutrophil phagocytosis and increased serum levels of Th1-derived pro-inflammatory cytokines such as TNFα, IFNγ and IL-2.120 In another study, subcutaneously infected NOD2-deficient mice showed a delayed and exacerbated ulcerative response, higher bacterial colony forming units (cfu) in the skin and impaired bacterial clearance when compared to NOD +/+ mice.121 Neutrophil numbers at the site of infection in both NOD2-deficient and NOD2 +/+ mice were similar and the authors attributed the observed action of NOD2, on IL-1β-amplified production of IL-16, which promoted rapid bacterial killing by neutrophils.121 The role of NOD signaling in killing of *S. aureus* has been nicely illustrated in a recent study of Clarke et al.122 Here it has been shown that priming of neutrophils through PGN via NOD1 signaling is a prerequisite for effective *S. aureus* killing. The authors postulate that bacterial flora is a source of peptidoglycan, which is essential for priming the innate immune system, which in turn ensures the killing of *S. aureus* via bone-marrow derived neutrophils. They demonstrate most elegantly that this priming occurs via NOD1, thereby linking NOD signaling with neutrophil mediated clearance of *S. aureus*. *S. aureus* can activate the NLRP3 inflammasome, another member of the NLR family which is linked to the activation of caspase-116,106 that is involved in the secretion of the pro-inflammatory cytokines IL-1β and IL-18.123

These studies demonstrate one very important aspect of NOD signaling, namely its effect on neutrophil mediated clearance of *S. aureus*. Therefore even though the recruitment of neutrophils is not impaired their functional capabilities with regard to *S. aureus* clearance are. Recent work shows that NOD1 signaling directly affects *S. aureus* clearance and that stimulation of NOD1 via PGN can positively regulate anti-*S. aureus* neutrophil reactivity.
Perspective

A large body of studies discussed in this review emphasize the importance of MyD88-dependent TLR2, IL-1R and NOD2 receptor signaling pathways triggered upon recognition by S. aureus-associated ligands. These early signals lead to the generation of various cytokines and chemokines cascades mediating the mobilization and recruitment of host inflammatory cells (notably neutrophils) at the infection sites but also appear to be specific for a particular site of infection. Clearly these pathways are responsible for the amplification and regulation of the inflammatory response, which leads either to the control of infection or exacerbated disease in certain types of inflammatory conditions.

An understanding of the precise molecular steps involved in these signaling pathways has implications for identifying potential target(s) for developing immunotherapeutic strategies to modulate events leading to enhanced neutrophil recruitment with increased anti-bacterial function at various staphylococcal infection sites. Because TLR2/IL-1R-MyD88 signals have been found to be crucial for generation of immunity against infection sites. Because TLR2/IL-1R-MyD88 signals have been found to be crucial for generation of immunity against S. aureus in a skin infection model, it has been proposed that this pathway can be used as a local therapeutic target such as in the topical administration of IL-1R or its mimetics. In the S. aureus lung model, the staphylococcal Protein A-TNFFR1 signaling pathway is activated (NFkB activation) in airway epithelial cells which mediate the recruitment of neutrophils resulting in bacterial clearance, but at the same time these cells exert deleterious effects on the airway epithelium contributing to the pneumonia. Thus, conceivably TNFFR1 could be used as potential target for strategies (such as the use of TNFFR1 antagonists) to modulate airway inflammation induced by S. aureus.

In several published studies, a contribution of TLRs other than TLR2 cannot be excluded. Thus, further studies are needed to reveal the redundant role of TLRs such as TLR9 in neutrophil mobilization at various sites of staphylococcal infection. TLR9 represents a MyD88-dependent TLR found in the intracellular endosomes and is reportedly recognized by staphylococcal DNA (CpG-DNA). In infections such as those caused by Mycobacterium tuberculosis or Trypanosoma cruzi, TLR2 and TLR9 are known to co-operate to control these infections.24,125

Studies using TLR2-/TLR9-double knockout mice are needed to clarify whether or not these receptors co-operate in controlling infections caused by S. aureus. The role of the NOD2-signaling pathway in S. aureus disease pathogenesis is not clear and warrants further investigations. Furthermore, knowledge about the activation of the cryopyrin/NLRP3 inflammasome in response to S. aureus infection is very limited.

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