Lipoproteins in Staphylococcus aureus infections

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Cover illustration: A battle between *Staphylococcus aureus* lipoproteins and the host immune cells (by Majd Mohammad)

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Knowledge is life and a cure

- Arabic proverb -

To my beloved family

ABSTRACT

Staphylococcus aureus (*S. aureus*) infections remain a major challenge for the healthcare system, and new treatment options are highly demanded. *S. aureus* is a pathogenic microorganism, responsible for a broad range of clinical infections in humans. Septic arthritis, a debilitating joint disease, is mainly due to *S. aureus*. Furthermore, the majority of skin and soft tissue infections are also caused by *S. aureus*. *S. aureus* expresses multiple bacterial molecules, including bacterial lipoproteins (Lpps), which play a role in the disease pathogenesis. *S. aureus* Lpps, the predominant ligands for TLR2, are important for bacterial survival due to their role in maintaining the metabolic activity of the bacteria. So far, their role in different staphylococcal infections have not been fully defined.

The aim of this thesis was to explore the role of *S. aureus* Lpp in the mouse models for septic arthritis and skin infection. The severity of septic arthritis and skin inflammation/infection as well as the molecular and cellular response of the host upon *S. aureus* Lpp exposure was the main focus of the thesis.

S. aureus Lpp, injected intra-articularly into murine knee joints, induced chronic macroscopic arthritis of a destructive character, which was mediated by monocytes/macrophages via TLR2. However, co-injection of purified *S. aureus* Lpp with *S. aureus* into mouse knees resulted in increased bacterial elimination. Mice intravenously infected with the *S. aureus* Lpp-expressing Newman parental strain, had increased mortality and weight reduction as well as impaired bacterial clearance in kidneys independent of TLR2 compared to those mice infected with Lpp-deficient strain. However, Lpp expression had no significant impact on the severity of bone destruction. Finally, in a skin infection model, expression of Lpp in *S. aureus* was associated with an

enhanced inflammatory response and increased bacterial burden in the local infection site.

In conclusion, *S. aureus* Lpps play differential roles depending on the route of infection. In the case of locally-induced arthritis, *S. aureus* Lpps play a dual role – on the one hand, Lpps contribute to joint inflammation and damage; on the other hand, Lpps elicit strong innate immune responses, resulting in efficient bacterial elimination. In haematogenous septic arthritis, Lpps have a limited impact on arthritis development. Finally, in the skin infection model, *S. aureus* Lpps contribute to local skin inflammation and enhance skin abscess formation.

Keywords: *Staphylococcus aureus*; lipoproteins; TLR2; septic arthritis; skin infection; mouse

SAMMANFATTNING PÅ SVENSKA

Trots stora framsteg inom hälso- och sjukvårdssektorn kvarstår stora utmaningar avseende bakterieinfektioner orsakade av gula stafylokocker (*Staphylococcus aureus*) och nya adekvata behandlingsmetoder är således högt efterfrågade. Denna bakterietyp förekommer bland omkring hälften av den friska vuxna befolkningen, vanligtvis i näsöppningen och som en del av hudfloran. Samtidigt är gula stafylokocker kända för att kunna vara aggressiva och är en ledande orsak till många kliniska infektioner, såsom led- och hudinfektioner samt livshotande blodinfektioner och sepsis. Till sitt förfogande har stafylokocker ett brett register av olika molekyler, inklusive lipoproteiner, som den använder till försvar mot vårt immunsystem. Bakteriella lipoproteiner uppfyller flera viktiga funktioner hos bakterien och är bland annat centrala för bakteriens överlevnad på grund av dess roll i att upprätthålla bakteriens metaboliska aktivitet. Lipoproteinernas specifika betydelse vid olika stafylokockinfektioner har däremot inte studerats väl.

I denna avhandling studerade jag lipoproteiners inverkan vid olika stafylokockinfektioner i musmodeller, och mer specifikt vid ledsjukdomen septisk artrit och vid hudinfektion. Interaktionen mellan stafylokocklipoproteiner och den efterföljande immunologiska responsen utforskades främst.

Sammantaget visar min avhandling att lipoproteiner i stafylokocker orsakar bestående och uttalad knäledsinflammation, till följd av ett överaktivt immunförsvar som angriper leden. Dessutom ger lipoproteinerna upphov till en ökad bakterieeliminering och minskad skada i knäleden i samband med att dessa blandas med levande bakterier genom att bidra till ökad aktivering av immunsystemet, och resulterar således i två helt olika fenomen. Vidare har jag även kunnat påvisa att stafylokocklipoproteiner ger en blodinfektion av svårare karaktär och slutligen till att dessa bidrar till framkallandet av hudinfektion med ökat inflammatoriskt svar samt hur bakterien tillämpar detta till sin fördel genom att skapa ett bättre skydd mot immunförsvaret.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Mohammad M**, Nguyen M-T, Engdahl C, Na M, Jarneborn A, Hu Z, Karlsson A, Pullerits R, Ali A, Götz F, Jin T.
The YIN and YANG of lipoproteins in developing and preventing infectious arthritis by *Staphylococcus aureus*.
PLoS Pathogens, 2019; 15(6): e1007877.

II. **Mohammad M**, Hu Z, Ali A, Kopparapu PK, Na M, Jarneborn A, Stroparo MN, Nguyen M-T, Karlsson A, Götz F, Pullerits R, Jin T. The role of *Staphylococcus aureus* lipoproteins in hematogenous septic arthritis.
Scientific Reports, 2020; 10(1):7936.

III. **Mohammad M**, Na M, Hu Z, Nguyen M-T, Kopparapu PK, Jarneborn A, Karlsson A, Ali A, Pullerits R, Götz F, Jin T.
The role of *Staphylococcus aureus* lipoproteins in skin infection.
Under revision

LIST OF PAPERS NOT INCLUDED IN THE THESIS

1. Jarneborn A, **Mohammad M**, Engdahl C, Hu Z, Na M, Ali A, Jin T. Tofacitinib treatment aggravates *Staphylococcus aureus* septic arthritis, but attenuates sepsis and enterotoxin induced shock in mice. *Sci Rep*. 2020; 10(1):10891.
2. Jin T, **Mohammad M**, Hu Z, Fei Y, Moore E, Pullerits R, Ali A. A novel mouse model for septic arthritis induced by *Pseudomonas aeruginosa*. *Sci Rep*, 2019; 9(1):16868.
3. Mitander A, Fei Y, Trysberg E, **Mohammad M**, Hu Z, Sakiniene E, Pullerits R, Jin T. Complement Consumption in Systemic Lupus Erythematosus Leads to Decreased Opsonophagocytosis *In Vitro*. *J Rheumatol*, 2018; 45(11):1557-1564.
4. Na M, **Mohammad M**, Fei Y, Wang W, Holdfeldt A, Forsman H, Ali A, Pullerits R, Jin T. Lack of Receptor for Advanced Glycation End Products Leads to Less Severe Staphylococcal Skin Infection but More Skin Abscesses and Prolonged Wound Healing. *J Infect Dis*, 2018; 218(5):791-800.
5. Baranwal G, **Mohammad M**, Jarneborn A, Reddy B, Golla A, Chakravarty S, Biswas L, Shankarappa S, Götz F, Jin T. Impact of cell wall peptidoglycan O-acetylation on the pathogenesis of *Staphylococcus aureus* in septic arthritis. *Int J Med Microbiol*, 2017; 307(7):388-397.
6. Fatima F, Fei Y, Ali A, **Mohammad M**, Erlandsson MC, Bokarewa MI, Nawaz M, Valadi H, Na M, Jin T. Radiological features of experimental staphylococcal septic arthritis by micro computed tomography scan. *PLoS One*, 2017; 12(2):e0171222.
7. **Mohammad M**, Na M, Welin A, Svensson MN, Ali A, Jin T, Pullerits R. RAGE Deficiency Impairs Bacterial Clearance in Murine Staphylococcal Sepsis, but Has No Significant Impact on Staphylococcal Septic Arthritis. *PLoS One*, 2016; 11(12):e0167287.
8. Ali A, Na M, Svensson M, Magnusson M, Welin A, Schwarze J, **Mohammad M**, Josefsson E, Pullerits R, Jin T. IL-1 Receptor Antagonist Treatment Aggravates Staphylococcal Septic Arthritis and Sepsis in Mice. *PLoS One*, 2015; 10(7):e0131645.
9. Ali A, Welin A, Schwarze JC, Svensson MN, Na M, Jarneborn A, Magnusson M, **Mohammad M**, Kwiecinski J, Josefsson E, Bylund J, Pullerits R, Jin T. CTLA4 Immunoglobulin but Not Anti-Tumor Necrosis Factor Therapy Promotes Staphylococcal Septic Arthritis in Mice. *J Infect Dis*, 2015; 212(8):1308-1316.

CONTENT

Abbreviations

ABC	ATP-binding cassette
agr	accessory gene regulator
ClfA, ClfB	clumping factor A, clumping factor B
Cna	collagen adhesin
Coa	coagulase
CP	capsular polysaccharide
ETs	exfoliative toxins
FnBPA	fibronectin binding protein A
FnBPB	fibronectin binding protein B
GC	guanine-cytosine
IL	interleukin
IRAK	interleukin-1 receptor-associated kinase
KC	keratinocyte chemoattractant
lgt	preprolipoprotein diacylglyceryl transferase
lit	lipoprotein intramolecular transferase
lns	lipoprotein *N*-acylation transferase system
lnt	apolipoprotein *N*-acyltransferase
Lol	localization of lipoprotein
lpl	lipoprotein-like
Lpl1	lipoprotein like protein 1
Lpps	lipoproteins
lsp	prolipoprotein signal peptidase
LTA	lipoteichoic acid
MAMPs	microbe associated molecular patterns
MCP-1	monocyte chemoattractant protein 1
MIP-2	macrophage inflammatory protein-2
MntC	manganese transport protein C

MRSA	methicillin-resistant Staphylococcus aureus
MSCRAMM molecule	microbial surface component recognizing adhesive matrix mole
MyD88	myeloid differentiation primary response gene 88
NF-κB	nuclear factor kappa B
NLRs	Nucleotide-binding oligomerization domain-like receptors
NOD	nucleotide-binding oligomerization domain-containing protein
OatA	*O*-acetyltransferase A
PGN	peptidoglycan
PRRs	pattern recognition receptors
PSMs	phenol-soluble modulins
Sak	staphylokinase
SE	staphylococcal enterotoxin
Sec	general secretory pathway
SEI	staphylococcal enterotoxin-like toxin
SitC	staphylococcal iron transport protein C
SpA	Staphylococcal protein A
SSL3	staphylococcal superantigen-like protein 3
TAK1	transforming growth factor β-activated kinase 1
Tat	twin arginine translocation
TIR	Toll/interleukin–1 receptor
TLRs	Toll-like receptors
TNF	tumour necrosis factor
TRAF6	tumour necrosis factor receptor–associated factor 6
TSS	toxic shock syndrome
TSST-1	Toxic shock syndrome toxin-1
vWbp	von Willebrand factor-binding protein
vWf	von Willebrand factor
WTA	wall teichoic acids

1. Introduction

Staphylococcus aureus (*S. aureus*) is mostly known as being associated with dreaded antibiotic-resistant infections, and rightly so, *S. aureus* plays a much broader role in human diseases. On the one hand, *S. aureus* functions as a commensal bacterium, colonizing nearly half of the human population, permanently or intermittently (Wertheim et al. 2005). On the other hand, *S. aureus* is a highly pathogenic microorganism with the ability to rapidly utilise its pathogenic properties as soon as it evades our body, and it frequently causes numerous severe clinical infections in humans, such as osteomyelitis, infective endocarditis, infectious arthritis, metastatic abscess formation and device-related infections (Edwards and Massey 2011; Tong et al. 2015). It is well-known that *S. aureus* is a very resourceful and dangerous pathogen in humans (Lowy 1998) and the leading cause of bloodstream infections (Edwards and Massey 2011). However, the mechanism how *S. aureus* transitions from colonization to infection remains elusive. Thus, gaining a greater understanding of its molecular mechanism and host-pathogen interaction is of vital importance in order to combat infectious diseases caused by *S. aureus*. Septic arthritis and skin infections, both typically caused by *S. aureus*, have been the focus of this thesis.

1.1 Septic arthritis

Septic arthritis, also known as infectious arthritis, remains one of the most dangerous and aggressive joint diseases due to its rapidly progressing and destructive nature, and is regarded as a potentially life-threatening condition (Rutherford et al. 2016). It is most commonly caused by *S. aureus* (Kaandorp et al. 1997), and it accounts for nearly 50% of all of the cases (Goldenberg 1998), while a variety of other bacterial, viral or fungal infections are also known to be implicated (Goldenberg 1998; Tarkowski 2006). Furthermore, *S. aureus* contributes to the most severe cases of infectious arthritis (Colavite and Sartori 2014), and is the most predominant cause of bacterial arthritis among rheumatoid arthritis and diabetic patients, counting for 80% of all of the cases (Goldenberg 1998). The current treatment alternatives are exactly the same as those used 30 years ago including antibiotics and drainage of the affected

joints. Lack of early or inadequate treatment can be fatal (Mathews et al. 2010). Even after initiation of immediate treatment, the joint damage caused by septic arthritis is often irreversible (Galloway et al. 2011). As a result of this debilitating disease, permanent joint dysfunction occurs in almost 50% of the patients (Kaandorp et al. 1995; Goldenberg 1998). Thus, joint replacement surgery is often needed.

Septic arthritis is an infectious disease that leads to an overwhelming inflammation in the joints (Colavite and Sartori 2014), and the determination of the joint destruction has been extensively studied for the past few decades (Mohammad et al. 2016; Mohammad et al. 2019; Mohammad et al. 2020; Tarkowski et al. 2001; Fei et al. 2011; Na et al. 2016; Ali, Na, et al. 2015; Ali, Welin, et al. 2015; Ali, Zhu, et al. 2015; Fatima et al. 2017; Jarneborn et al. 2020; Jin et al. 2019). Interplay between the bacterial virulence factors and host-immune response is considered to be decisive and of high pathogenic importance during the progress of the disease (Colavite and Sartori 2014). The disease course is characterized by an exaggerated immune response with rapid influx of monocytes and neutrophils, and substantial activation of macrophages in the local infection site (Bremell, Abdelnour, and Tarkowski 1992; Verdrengh et al. 2006), subsequently leading to pannus formation and destruction of both cartilage and bone (Colavite and Sartori 2014).

The incidence of septic arthritis is estimated to be 4-10 cases per 100,000 individuals in the general population per year (Tarkowski 2006; Mathews et al. 2010). Two age groups that are particularly susceptible of acquiring septic arthritis are the paediatric population under 15 years of age as well as the population over 55 years of age (Nade 2003). However, the frequency of septic arthritis in patients with a pre-existing joint disease, such as rheumatoid arthritis, or with implants of prosthetic joints, is up to 10 times higher compared to the general population (Tarkowski 2006). Thus, the indisputably leading risk factors for the onset of septic arthritis are pre-existing joint disease as well as orthopaedic joint surgery (Colavite and Sartori 2014; Tarkowski 2006). Other risk factors that predispose to infectious arthritis include increased age, recent surgical intervention of the joint, skin infection, immunosuppressive treatment or immunodeficiency, intravenous drug abuse and diabetes mellitus (Kaandorp et al. 1995). For example, in rheumatoid arthritis, the mortality rate among patients suffering from monoarthritis (infection in one single joint) corresponds to 19% (Tarkowski 2006). However,

in terms of polyarthritis, a more serious manifestation of septic arthritis and a sign of undesirable outcome, the mortality rate amounts to approximately 50% (Tarkowski 2006).

Originating from another infection site such as abscess or pneumonia, bacterial dissemination through the bloodstream into the joints is the most common route of acquiring septic arthritis (Goldenberg and Reed 1985). *S. aureus* is very prone to escape the immune system and enter the joint cavity. Larger peripheral joints are more susceptible to infection (Margaretten et al. 2007), with the knee joint accounting for approximately half of the staphylococcal septic arthritis cases in humans (Goldenberg 1998). In a mouse model, *S. aureus* is known to induce severe bone erosion within a couple of days after infection upon intravenous inoculation (Fatima et al. 2017). However, the exact mechanism that haematogenously spreading *S. aureus* employs to escape various immunological checkpoints in the bloodstream before it can cross the endothelial barriers along the way, and before it is able to establish new infection sites, is still wrapped in mystery (Edwards and Massey 2011). *S. aureus* can also directly enter the joint cavity and induce septic arthritis without dissemination through the blood, e.g. due to direct trauma to the joints, during intra-articular injections or during prosthetic surgical interventions (Goldenberg and Reed 1985), although this is much less common (Shirtliff and Mader 2002).

1.2 *S. aureus* cutaneous infections

The human skin, functioning as the largest organ in the body (Grice and Segre 2011), serves as a critical first line of host defence by which its physical barrier efficiently shields against invading pathogens (Kobayashi, Malachowa, and DeLeo 2015). The skin along with its underlying soft tissue, which represents the majority of the tissue in the body (Dryden 2009), are consistently exposed to traumatic wounds or ruptures, which subsequently increases the risk of skin and soft tissue infections in humans (Dryden 2009). Skin and soft tissue infections belong to a category of infections which are not only ubiquitous, but also very common, with most of the population affected at some time point in their life span (Dryden 2010). Although *S. aureus* may reside harmlessly on the skin, this microorganism has an immense pathogenic potential and can become intensively deleterious under the right circumstances. In fact, *S. aureus*

is well-known to be the most prominent microorganism isolated from skin and soft tissue infections (Miller and Cho 2011; McCaig et al. 2006), and encompasses a broad spectrum of clinical manifestations, ranging from minor and superficial infections to life-threatening conditions (Dryden 2009). Classical examples of skin and soft tissue infections are infected ulcers and wounds, folliculitis, cellulitis and impetigo (Miller and Cho 2011). More severe forms of *S. aureus* skin infections may transform to bacteraemia, e.g. the presence of bacteria in the bloodstream, potentially leading to the infection in different organs such as lungs (pneumonia), membranes surrounding the brain (meningitis), heart valves (endocarditis) and sepsis (Miller and Cho 2011; Lowy 1998). *S. aureus* is notorious for skin abscess formation (Krishna and Miller 2012a), and thus to understand the underlying mechanism behind this phenomenon has been one of the goals of this thesis.

1.3 *S. aureus* and the threat of antibiotic resistance

The fierce combat against *S. aureus* and its increasing resistance proceeds with enhanced global spread and failure of promoting new successful therapeutic alternatives remain. This consequently results in severe clinical and economic burdens for societies worldwide. As *S. aureus* possesses an extraordinary capability to acquire antibiotic resistance (DeLeo et al. 2010), the spread of *S. aureus* antibiotic-resistant strains and its attributable infections has reached epidemic levels worldwide (Chambers and Deleo 2009; Grundmann et al. 2006). In fact, the emergence of *S. aureus* resistant strains, including methicillin-resistant *S. aureus* (MRSA), is strongly associated with greater incidence and increasingly complicated *S. aureus* infections of more invasive nature, which significantly limits the adequate treatment options (Tong et al. 2015; DeLeo et al. 2010). In addition, the most severe MRSA infections are no longer strictly restricted to hospital intensive care or acute care units, as invasive MRSA infections are highly distributed on a considerably broader area (Klevens et al. 2007). Furthermore, infections associated to MRSA are attributed to both hospital environments as well as to the communities, because these can be acquired in both areas – commonly known as healthcare-associated MRSA and community-associated MRSA, respectively. This simply puts not only hospitalised patients at risk but the general and otherwise healthy population as well (DeLeo et al. 2010).

Of note, *S. aureus* has been identified as the most prominent cause of infections within the health care facilities in the United States, whereby MRSA, in particular, accounts for a high proportion of these infections (DeLeo and Chambers 2009). Importantly, MRSA is now considered as one of the primary causes of increased mortality triggered by a single pathogen (DeLeo and Chambers 2009). It is therefore of vital importance to emphasise the magnitude of MRSA infections and the consequences they give rise to from a global point of view. Hence, better understanding of the underlying mechanisms behind the virulent functions of *S. aureus* infections and its ability to effectively acquire resistance may lead to improved and urgently needed therapies. The development of effective prophylactic vaccines against *S. aureus* infections is an appealing thought, although no attempts have succeeded so far, most likely due to the lack of a clear virulent target in *S. aureus* (Proctor 2012). Immunotherapy has caught the attention of researchers in recent years and is indeed an exciting and promising area of research. By harnessing the immune system through targeted therapies, an increased host cellular response may both promote elimination of invading pathogens as well as pave the way for fundamentally novel therapeutic modalities with the goal to overcome the immense challenge caused by the rapid emergence of antibiotic-resistant bacteria.

Importantly, sub-inhibitory concentrations of antibiotics promote the release of extracellular DNA in *S. aureus* and the induction of biofilm formation (Kaplan et al. 2012). Furthermore, in previous studies, sub-minimal inhibitory levels of β-lactam antibiotics have been shown to upregulate expression of various *S. aureus* virulence factors (Dumitrescu et al. 2011; Kernodle et al. 1995; Stevens et al. 2007; Shang et al. 2019). Overall, it is of outmost importance to use the antibiotics accurately during infections – both in terms of the selection of the correct drug and adequate/optimal dosage, as well as early treatment initiation at the onset of infection.

2. *S. aureus* and its virulence factors

S. aureus is a Gram-positive bacterium that possesses an immense arsenal of virulence factors, which enable the bacterium to exercise its perilous potential in order to thrive as an opportunist in humans. Some of the virulence factors deployed by *S. aureus* are described below and depicted in Figure 1.

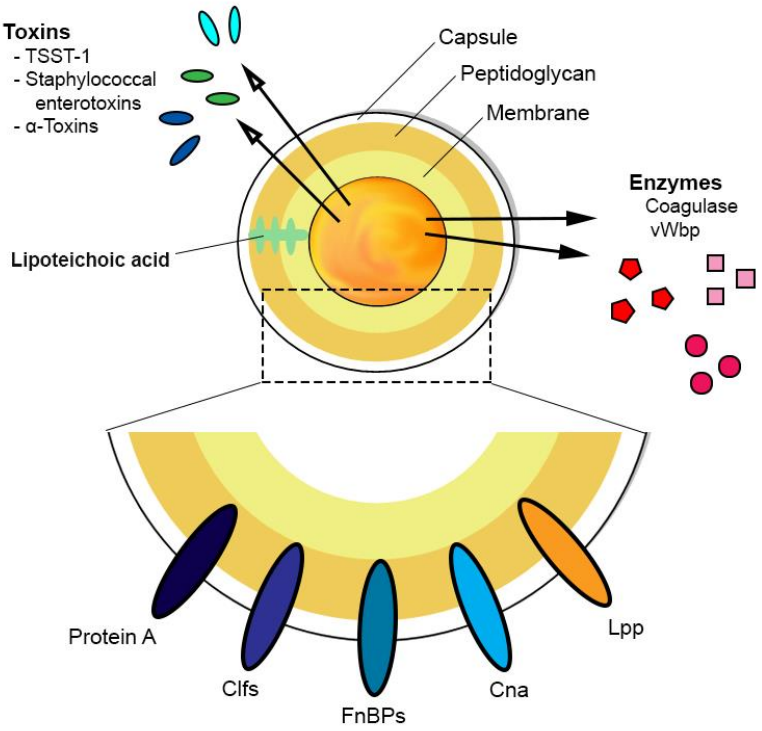


Figure 1. Schematic diagram illustrating the basic structure of Staphylococcus aureus and its ability to express various virulence factors. *TSST-1 = Toxic shock syndrome toxin-1, Clfs = clumping factors, FnBPs = fibronectin binding proteins, Cna = Collagen adhesin, Lpp = lipoprotein, vWbp = von Willebrand factor-binding protein.*

2.1 Cell wall components

The architecture of the cell wall and cell envelope in *S. aureus* has a complex nature. The cell wall comprises a multi-layered structure with **peptidoglycan** (PGN) as the main component (Lowy 1998). PGN is known for its characteristically heterogeneous structure and is composed of cross-linked polymers arranged with alternating N-acetylglucosamines and N-acetylmuramic acid sugar residues with β-1,4 linkage formations (Giesbrecht et al. 1998; Lowy 1998), corresponding to 20 – 30 nm in thickness (Sharif et al. 2009). The cross-linked structure is established through peptide bridges consisting of L- and D-amino acids, which form its 3-dimensional structure that surrounds the entire bacterium (Lowy 1998). In fact, PGN covers up to half of the total weight of the cell wall of *S. aureus* (Lowy 1998), and serves a critical role in upholding the structural integrity of the bacterium as well as providing a protective shield against the host (Sorbara and Philpott 2011). Through detection by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), PGN highly alerts the innate immune system upon exposure, as it serves as a microbe-associated molecular pattern (MAMP) component (Sorbara and Philpott 2011).

S. aureus PGN has previously been shown to induce arthritis in a local mouse knee joint model (Liu et al. 2001). However, this is in contrast to our recent study where we demonstrated that purified *S. aureus* PGN only gave rise to mild and transient macroscopic arthritis (**Paper I**) (Mohammad et al. 2019). These contradicting findings can be attributed to differences in administered doses of PGN or contamination by lipoproteins (Lpps), which is discussed in more detail below.

Besides, secondary modifications of the PGN cell wall are of vital importance in order to resist host immune response enforcements (Bera et al. 2005). Antibacterial enzymes produced by the host, such as lysozyme, also known as muramidase, can cleave PGN in the β-1,4 linkage site localised between the sugar residues of N-acetylglucosamine and N-acetylmuramic acid, thereby inhibiting bacterial overgrowth (Bera et al. 2005; Keshav et al. 1991). However, *S. aureus* impressively protects itself from 'cell wall breakdowns' by utilizing *O*-acetylation modifications mediated by the *O*-acetyltransferase A (*OatA*) enzyme in the PGN cell wall (Sychantha et al. 2017). We

demonstrated that the virulence associated with PGN *OatA*, in both systemic and local *S. aureus*-induced septic arthritis, showed milder progression of the disease in mice infected with a Δ*oatA* mutant strain (Baranwal et al. 2017), indicating the pathogenic importance of the *O*-acetylation of PGN in staphylococcal septic arthritis.

Among the constituents of the cell wall of *S. aureus* are the **teichoic acids**, made up of polyribitol- and polyglycerol phosphates as well as sugar-containing polymers (Reichmann and Grundling 2011; Brown, Santa Maria, and Walker 2013). Teichoic acids are not unique to *S. aureus* but are found in various Gram-positive bacteria. They can be categorised into wall teichoic acids (WTA) that are covalently attached to the bacterial PGN, and **lipoteichoic acids** (LTA) that are anchored to the lipid membrane through a glycolipid (Reichmann and Grundling 2011; Brown, Santa Maria, and Walker 2013).

Historically, LTA has been regarded as a potent stimulator of the innate immunity upon its recognition by TLR2 among others, leading to the activation of the macrophages and the release of pro-inflammatory cytokines (Morath, Geyer, and Hartung 2001; Kusunoki et al. 1995; Morath et al. 2002). However, recent studies cast a shadow of doubt on these results, pointing a finger to contamination by other cell wall components of *S. aureus*, discussed further below.

WTA has been implicated in the pathogenesis of *S. aureus*-induced endocarditis (Weidenmaier et al. 2005) and endophthalmitis (Suzuki et al. 2011). LTA, on the other hand, has been shown to damage the skin barrier, inducing skin inflammation (Brauweiler, Goleva, and Leung 2019) and elevated levels of LTA were recovered in children with infected atopic dermatitis lesions (Travers et al. 2010). In addition, LTA is essential in shielding the bacteria against antimicrobial peptides (Peschel et al. 1999).

Capsular polysaccharides (CP) surround the cell wall of *S. aureus*. So far, more than 10 different serotypes of CP have been described, although two serotypes, CP5 and CP8 constitute the main serotypes isolated from clinical strains (Nanra et al. 2013; Mohamed et al. 2019). One of the functions of the CP is to protect the bacteria from the innate immune system by evading complement binding and subsequent killing by phagocytes (Guidry et al. 1991;

O'Riordan and Lee 2004). In addition, Nilsson *et al*. showed that the CP5 serotype is a virulence factor in *S. aureus* induced septic arthritis and sepsis (Nilsson et al. 1997). Furthermore, conjugate vaccine of CP8 but not CP5 conferred protection against *S. aureus* induced dermonecrotic skin lesions in murine skin infection model (Cheng et al. 2017).

2.2 Lipoproteins

Among the wide array of bacterial molecules that *S. aureus* exhibits are the **lipoproteins** (Lpps), which is the main focus of this thesis.

The aims of the studies in this thesis were to understand the role of *S. aureus* Lpps in infections, more specifically:

1. Determine whether *S. aureus* Lpps are arthritogenic, and if so, to elucidate the molecular and cellular mechanism behind it (**Paper I**),
2. Investigate the importance of *S. aureus* Lpp and TLR2 in murine haematogenous septic arthritis (**Paper II**),
3. Study the role of *S. aureus* Lpp in skin infections (**Paper III**).

S. aureus Lpp consists of a lipid-moiety and a protein-moiety. The lipid portion is covalently attached to a cysteine residue in the N-terminal region, ultimately facilitating its anchoring in the outer leaflet of the bacterial cytoplasmic membrane (Nguyen and Gotz 2016). In contrast to *S. aureus* and other Gram-positive bacteria, bacterial Lpps are also lipid-anchored in the inner leaflet of the outer membrane in Gram-negative bacteria (Braun and Rehn 1969). Bacterial Lpps were first discovered a few decades ago in *Escherichia coli*, and named Braun's lipoprotein after its discoverer, as an *S*-glyceryl-cysteine residue was detected with modifications comprising a trio of fatty acids at its N-terminal region (Hantke and Braun 1973). This finding was considered as a paradigm and as the starting point for research related to bacterial Lpp. Lpp and its functions are discussed in detail below.

2.3 Accessory gene regulator (*agr*)

The pathogenicity of *S. aureus* depends on the diversity of virulence factors it expresses and secretes such as surface proteins, toxins and enzymes. To establish and maintain infections, the bacterium must regulate its virulence factors and to deploy the right virulence factors at the right time. For example, to establish an infection, the bacterium first needs to adhere to the host's tissues and organs and once it establishes an infection, the next step is invasion and further spreading to tissues and organs.

The majority of virulence factors of *S. aureus*, of which many are described below, are regulated by the *agr* quorum sensing system (Wang and Muir 2016). The *agr* system is composed of four genes termed *agrA* – *agrD* (Vuong et al. 2000). The *agr* system functions by sensing the cell density of the bacteria, from the exponential- to the stationary growth phase. This is made possible by quorum sensing – a cell to cell communication mechanism employed by the bacteria – which produces the autoinducer that will guide the bacteria towards to a decision when and which virulence genes to upregulate or downregulate (Wang and Muir 2016). Therefore, it is of no surprise that *agr* positive strains are pathogenic in different *S. aureus* infections, such as skin infections (Kobayashi et al. 2011; Cheung et al. 2011; Kennedy et al. 2010), septic arthritis (Nilsson et al. 1996; Abdelnour et al. 1993), endocarditis (Cheung et al. 1994; Powers et al. 2012), and pneumonia (Bubeck Wardenburg, Patel, and Schneewind 2007). Furthermore, the disruption of the *agr* signalling ameliorates the spread of *S. aureus* infections; hence *agr* has been touted as a possible drug target (Otto 2004; Wang and Muir 2016).

In this thesis, both *agr* positive (Newman) as well as *agr* negative (SA113) strains (Herbert et al. 2010) have been used.

2.4 Surface proteins

Another category of virulence factors possessed by *S. aureus* is the expression of surface proteins (Foster et al. 2014). These bacterial surface proteins, also known as cell wall-anchored proteins, are covalently linked to the cell wall PGN facilitated by sortase-transpeptidase enzymatic reactions (Foster et al. 2014; Lacey et al. 2017; Hendrickx et al. 2011). A distinguishing feature

among these proteins is a sorting signal domain, featuring a LPXTG motif, which enables the enzymatic cleavage to be carried out (Schneewind, Model, and Fischetti 1992), ultimately giving rise to a broad range of virulent proteins on the surface of the bacterium (Mazmanian et al. 2002; Cheng et al. 2009). So far, more than 20 different cell wall-anchored proteins expressed by *S. aureus* have been identified (Foster et al. 2014; Foster 2019), although the number varies among different *S. aureus* strains (Foster et al. 2014; McCarthy and Lindsay 2010). These specific proteins play a key role in the survival of the bacterium, as they behave in an adhesive, invasive and evasive manner towards the host's tissues and immune response (Foster et al. 2014). The largest class of the cell wall-anchored proteins is the **microbial surface component recognizing adhesive matrix molecules** (MSCRAMMs), which facilitate a linkage between the microbe and the fibronectin, fibrinogen and collagen of the host extracellular matrix, and have a fundamental role in the initial stage in the pathogenesis of most *S. aureus* infections (Patti, Allen, et al. 1994). Clumping factors, Staphylococcal Protein A, fibronectin binding proteins, are important members of the MSCRAMMs family.

Clumping factor A (ClfA) is known to favour adhesion to fibrinogen in the blood plasma as well as to mediate microbial attachment to indwelling devices. This usually promotes biofilm formation, and subsequently enables *S. aureus* to colonize and establish a solid and stable infection evading the immune response mounted by the host. Of note, the primary effect of ClfA appears to occur at the early stage of infection (Cheng et al. 2009).

The role of ClfA in various *S. aureus* infections is significant. In a murine septic arthritis model, inoculation with a ClfA mutant *S. aureus* strain clearly reduced the arthritogenicity as well as the mortality rate of mice compared to its parental *S. aureus* Newman strain (Josefsson et al. 2001). Another study showed that a deletion mutant of ClfA contributed to less formation of skin abscesses in a rabbit model (Malachowa et al. 2016), as well as exhibited impaired virulence in mouse skin tissue (Kwiecinski, Jin, and Josefsson 2014).

Similar to ClfA, ClfB is a fibrinogen binding protein and resembles ClfA with regard to its sequence, although ClfB binds to different parts of the fibrinogen (Ni Eidhin et al. 1998). Naturally, colonization and subsequent bacterial infection of the skin tissue is initiated by adhesion of the bacteria to the corneocytes, more specifically the cornified envelope, which surrounds the

corneocytes (Fleury et al. 2017). ClfB has been shown to particularly promote adhesion of *S. aureus* in the nares therefore facilitating colonization with consequent skin infections (Lacey et al. 2019). Hence, ClfB has been implicated in several staphylococcal skin tissue infections (Fleury et al. 2017; Lacey et al. 2019). Lacey *et al*. demonstrated that ClfB could be a vaccine target in *S. aureus* skin and soft tissue infections, inducing both humoral and cellular responses (Lacey et al. 2019). ClfB has also been shown to be pathogenic in *S. aureus* experimental endocarditis, albeit to a lesser degree than ClfA (Entenza et al. 2000).

Moreover, we recently assessed whether *S. aureus* Lpp contributes to enhanced ClfA or ClfB levels in comparison to its Lpp deficient strain, but observed that Lpp have no impact on the expression levels of these specific virulence factors (**Paper II**) (Mohammad et al. 2020).

Staphylococcal protein A (SpA), a major *S. aureus* surface protein, was the first to be discovered of the surface proteins (Jensen 1958; Mazmanian, Ton-That, and Schneewind 2001). Hitherto, all *S. aureus* clinical isolates contain the SpA gene that codes for the protein A (Votintseva et al. 2014). SpA is notable for its immune evasion capabilities. It effectively impedes antibody recognition by binding to the Fc region of IgG in both humans and mice (Falugi et al. 2013).

Abscess formation is a hallmark of staphylococcal skin infections (Cheng et al. 2011; Kobayashi, Malachowa, and DeLeo 2015) and SpA has been found to play an important role in skin abscess formation (Cheng et al. 2011). The ability to form skin abscess was shown to be diminished in staphylococcal mutants lacking SpA compared to their wild-type counterparts (Cheng et al. 2009; Kwiecinski, Jin, and Josefsson 2014). In patients with atopic dermatitis, expression of SpA was more common and was associated with aggravated dermatitis lesions (Yao et al. 2010). In murine septic arthritis model, administration of bacteria expressing SpA exacerbated not only arthritis but also significantly increased the mortality of mice compared to the mutant strains lacking the SpA gene (Palmqvist et al. 2002). Interestingly, the level of SpA expression was somehow regulated by *S. aureus* Lpp (**Paper II**) (Mohammad et al. 2020). Total RNA extract from Lpp expressing *S. aureus* strain showed elevated transcriptional levels of SpA compared to the Lpp

deficient *lgt* mutant strain, in early, but not at late hours of bacterial culturing (**Paper II**) (Mohammad et al. 2020).

Another member of the MSCRAMM family is the **collagen adhesion** (Cna) that binds to collagen, the most abundant protein in the human body and a key structure of the connective tissues (Madani, Garakani, and Mofrad 2017). Cna facilitates the adhesion of *S. aureus* to cartilage (Xu et al. 2004), and as a result aggravates *S. aureus*-induced septic arthritis (Xu et al. 2004; Patti, Bremell, et al. 1994) and osteomyelitis (Elasri et al. 2002) in murine models. However, contradictive findings have been reported regarding the virulence of Cna in septic arthritis and osteomyelitis in patients (Thomas et al. 1999; Ryding et al. 1997; Switalski et al. 1993).

As mentioned above, **Fibronectin binding protein A** (FnBPA) and **Fibronectin binding protein B** (FnBPB) are members of the MSCRAMM family. Both FnBPA and FnBPB bind to fibrinogen, fibronectin and elastin (Pietrocola et al. 2019; Roche et al. 2004; Wann, Gurusiddappa, and Hook 2000). Similar to several other members of the MSCRAMM family, the FnBPs promote adhesion of *S. aureus* to host cells (Foster et al. 2014). In addition, both FnBPs act as invasins, facilitating the internalization of the bacterium into epithelial cells (Dziewanowska et al. 1999).

The FnBPs have been found to promote abscess formation in *S. aureus* skin infections (Kwiecinski, Jin, and Josefsson 2014). Abscess formation was diminished in mice inoculated with staphylococcal strains lacking the FnBPs compared to mice inoculated with its parental strain (Kwiecinski, Jin, and Josefsson 2014). With regard to septic arthritis, FnBPs have been found to be of less importance in development of arthritis (Palmqvist et al. 2005). However, on the other hand, FnBPs are crucial for inducing systemic inflammation leading to significant more weight loss and higher mortality in a murine model (Palmqvist et al. 2005). Also, FnBPB expression is more closely associated with endocarditis infection than with arthritis or osteomyelitis (Tristan et al. 2003).

2.5 Secreted molecules

Most clinical *S. aureus* isolates secrete **coagulase** (Coa) and hence expression of Coa is used clinically to discriminate *S. aureus* from other staphylococcal species (McAdow, Missiakas, and Schneewind 2012; Lowy 1998). Coa promotes clotting of blood by binding to and activating prothrombin which will convert fibrinogen to fibrin (Cheng et al. 2010; Lowy 1998), an evasion mechanism by *S. aureus* to shield itself from phagocytosis. In *S. aureus* induced osteomyelitis, Coa aggravates bone loss as well as bone destruction by inhibiting the proliferation of osteoblasts (Jin et al. 2013).

Von Willebrand factor-binding protein (vWbp) is a bacterial enzyme secreted by *S. aureus* and similar to Coa, is involved in host's clot formation by activating prothrombin that in turn cleaves fibrinogen to fibrin (Cheng et al. 2010; McAdow, Missiakas, and Schneewind 2012). Additionally, as its name suggests, vWbp acts as a bridge between the bacterial cell wall and von Willebrand factor (vWf) therefore enabling adhesion of *S. aureus* to vascular tissues (Claes et al. 2014; Cheng et al. 2011; Claes et al. 2017).

In contrast to Coa and vWbp that promote formation of fibrin clots, the **staphylokinase** (Sak) protein produced by *S. aureus* has the opposite role, namely, activating plasminogen to form plasmin that leads to the digestion of fibrin clots. Thus, *S. aureus* directly influences the host fibrinolytic system using it to its advantage to spread to new tissues (Molkanen et al. 2002). In *S. aureus* skin infections, dual roles of Sak were described. On the one hand, Sak facilitated establishment of infection, while on the other hand, it attenuated disease severity by promoting drainage of the formed abscesses (Kwiecinski et al. 2013). Furthermore, activation of the host's plasminogen by Sak attenuated *S. aureus* systemic infection in mice (Kwiecinski et al. 2010).

2.6 Toxins

One of the properties that make *S. aureus* such a formidable threat is the vast amount of toxins it secretes. Toxins produced by *S. aureus* are usually categorised into three different groups depending on their functions; superantigens, cytotoxins and exfoliative toxins (Oliveira, Borges, and Simoes 2018). *S. aureus* produces quite many superantigens that fall into three

categories, **staphylococcal enterotoxins** (SEs), **staphylococcal enterotoxin-like toxins** (SEls) and **toxic shock syndrome toxin-1** (TSST-1) (Xu and McCormick 2012).

The SEs, in turn consist of SEs A, B, C, D, E, G, H, I, R and T whereas the SEIs consist of J, K, L, M, N O, P, Q, S, U, V, and X (Xu and McCormick 2012; Argudin, Mendoza, and Rodicio 2010). Both, the SEs and SEIs, have quite similar structures. The main difference is that the SEs are able to induce emesis that leads to diarrhoea and vomiting associated with food poisoning (Xu and McCormick 2012), whereas SEIs generally do not (Xu and McCormick 2012; Argudin, Mendoza, and Rodicio 2010).

TSST-1 released by *S. aureus* can cause the potentially fatal toxic shock syndrome (TSS) that is characterized by fever, rash and low blood pressure rapidly progressing to shock with multiple organ failure (Lappin and Ferguson 2009). TSST-1 prompts the major histocompatibility complex (MHC)-II on antigen-presenting cells to bind non-specifically to receptors of the T-cells, circumventing the normal antigen-presenting phase and leading to massive activation of T-cells (Fraser 2011). In fact, certain studies have shown that up to one fifth of all the T-cells can be activated in patients with TSS (Rodstrom, Elbing, and Lindkvist-Petersson 2014). This is followed by a cytokine storm with enormous release of various cytokines and chemokines (Low 2013; Kong, Neoh, and Nathan 2016). The majority of non-menstrual TSS cases as well as all menstruation-associated TSS are caused by TSST-1 (McCormick et al. 2003).

In haematogenous *S. aureus* septic arthritis model, TSST-1 was shown to aggravate the disease severity (Abdelnour, Bremell, and Tarkowski 1994). Mice intravenously injected with TSST-1 secreting *S. aureus* developed more frequent and severe arthritis compared to mice inoculated with TSST-1 deficient *S. aureus* (Abdelnour, Bremell, and Tarkowski 1994). However, direct injection of purified TSST-1 into mouse knee joint failed to induce joint inflammation (**Paper I**) (Mohammad et al. 2019).

Thus far, four different **exfoliative toxins** (ETs) released by *S. aureus* have been identified, named ETA – ETD (Oliveira, Borges, and Simoes 2018). The exfoliative toxins, especially A and B released by *S. aureus*, are responsible for causing staphylococcal scalded skin syndrome (Leung, Barankin, and

Leong 2018; Handler and Schwartz 2014). The syndrome, occurring mostly in children, is associated with blistering of the skin and is considered a paediatric emergency. It can be fatal, especially in adults (Leung, Barankin, and Leong 2018; Handler and Schwartz 2014).

Among the arsenal of toxins secreted by *S. aureus* are the pore forming, cell-membrane damaging cytotoxins including the haemolysins, bi-component leucocidins and the phenol-soluble modulins.

The **haemolysins**, made up of α, β, and γ haemolysins, are pore-forming toxins that cause lysis of the red blood cells. Of these, the α-haemolysin, even referred to as the α-toxin, is the most studied and has been shown to cause lysis of several type of mammalian cells, not only the red blood cells (Bhakdi and Tranum-Jensen 1991). The α-haemolysin has been implicated in the pathogenesis of several *S. aureus* caused diseases including septic arthritis (Nilsson et al. 1999), skin diseases (Walev et al. 1993; Hong et al. 2014), pneumonia (Kebaier et al. 2012), and sepsis (Cremieux et al. 2014).

The **bi-component leucocidins** target the leukocytes, especially the phagocytes, thus preventing the elimination of *S. aureus* by the host's immune cells (Spaan, van Strijp, and Torres 2017; Alonzo et al. 2012). Chief among them is the **Panton-Valentine Leucocidin**, even known as the LukSF-PV, which is overrepresented in *S. aureus* isolates that cause necrotic skin lesions (Adler et al. 2006).

The **phenol-soluble modulins** (PSMs) consist of small peptides and similar to the haemolysins and leucocidins have pore-forming properties. The PSMs can target several cell types such as erythrocytes and leukocytes and have been shown to induce inflammation (Cheung et al. 2014). Members of this family include the PSM-mec, PSMα 1-4, PSMβ 1-2 as well as PSMγ (Cheung et al. 2014; Qin et al. 2016). Apart from the role in biofilm structuring and dispersal (Periasamy et al. 2012; Cheung et al. 2014; Peschel and Otto 2013), PSMs facilitate invasion and killing of osteoblasts thereby aggravating *S. aureus*-induced osteomyelitis (Rasigade et al. 2013).

3. The immune response in infections caused by *S. aureus*

S. aureus, through its vast virulence factors, seeks multiple ways to colonize and establish infections in humans. However, upon intrusion, this pathogenic bacterium highly alerts the host's immune system. Consequently, a battle between the host and the pathogen starts. Some of the complications and immune responses that arises upon the host-pathogen interactions during septic arthritis and skin infection are briefly discussed in this chapter.

3.1 Innate immunity

The host's innate immune system immediately executes a series of protective measures against intruding pathogens, such as *S. aureus*, and this serves as the first line of defence (Akira, Uematsu, and Takeuchi 2006). This action is initially implemented through recognition via PRRs that distinctively sense pathogenic components and promptly trigger the activation of innate immune cells (Akira, Uematsu, and Takeuchi 2006). Among these immune cells are the phagocytes.

3.1.1 Neutrophils and macrophages

The most abundant type of the leukocytes are the polymorphonuclear neutrophils, making up to 50-70% of the white blood cells in humans (Mestas and Hughes 2004). Neutrophils are a subset of granulocytes and an indispensable part of the innate immunity. Neutrophils are under normal conditions confined in the bloodstream and can be quickly recruited to tissues and organs upon *S. aureus* infection through chemotaxis. They play a critical role in eliminating the bacteria (Kolaczkowska and Kubes 2013). PRRs resided on the surface of the neutrophils facilitate the detection of *S. aureus* and subsequently opsonisation and phagocytosis (van Kessel, Bestebroer, and van Strijp 2014). Phagocytosis of the bacteria is followed by the release of reactive oxygen species and antimicrobial peptides by the neutrophils that result in degradation and ultimately elimination of the pathogen (Guerra et al. 2017).

Macrophages are, unlike the neutrophils, mononuclear and account for less than 10% of the leukocytes. The precursors to macrophages are the monocytes that circulate in the blood and which upon activation can differentiate to macrophages. The macrophages are the main source of numerous important cytokines and chemokines that play an important part of the immune response. Depending on their point of entry to the body, pathogens would first encounter tissue resident macrophages that are usually confined to specific organs such as Kupffer cells in the liver, osteoclasts in the bones or alveolar macrophages in the lungs (Kierdorf et al. 2015).

As expected, macrophages were shown to be essential in bacterial elimination, thus ridding the body of the invading pathogen (Verdrengh et al. 2006). However, it has also been demonstrated that macrophages aggravate the arthritis severity in a murine model of live *S. aureus*-induced septic arthritis (Verdrengh et al. 2006), potentially due to enhanced secretion of the pro-inflammatory cytokine, TNFα (Hultgren et al. 1998). In the case of arthritis induced by antibiotic-killed *S. aureus*, a close crosstalk between macrophages and neutrophils is necessary for induction of joint inflammation (Ali, Zhu, et al. 2015).

We recently demonstrated that purified *S. aureus* Lpp rapidly initiates the recruitment of monocytes/macrophages and neutrophils upon local knee injection (**Paper I**) (Mohammad et al. 2019). A similar outcome with influx of inflammatory cells was also observed in the skin model (**Paper III**). Yet, leukocyte depletion was shown to diminish the Lpp-induced effect (**Paper III**). In the model of local knee arthritis induced by purified *S. aureus* Lpp, depletion of monocytes/macrophages resulted in diminished bone destruction, whereas neutrophil depletion played a minor role (**Paper I**) (Mohammad et al. 2019). This demonstrates that monocytes/macrophages are the key cell types in the development of local knee arthritis induced by purified Lpl1. Importantly, when purified Lpl1 and live *S. aureus* were co-injected into murine knee joints, bacterial eradication occurred. This was mediated through monocytes/macrophages and mainly neutrophils, since depletions of these phagocytes resulted in aggravated disease severity and increased bacterial burden in local knee joints (**Paper I**) (Mohammad et al. 2019). Although *S. aureus* Lpps are known as potent stimulators of nitric oxide synthase and mediate nitric oxide production in mouse macrophages (Kim et al. 2015), our data showed that anti-nitric oxide synthase treatment had no impact on the

bacterial clearance in knee joints (**Paper I**) (Mohammad et al. 2019). In the haematogenous *S. aureus*-induced septic arthritis model, neutrophils are known to mediate critical protection as neutrophil-depleted mice exhibited more severe disease (Verdrengh and Tarkowski 1997). In addition, in the *S. aureus* skin infection model, Mölne *et al*. demonstrated that neutrophil depletion worsens the disease severity with increased bacterial burden in the skin tissue of mice (Molne, Verdrengh, and Tarkowski 2000). These results indicate that neutrophils play a critical protective role in both local as well as systemic arthritis and *S. aureus* skin infections (Mohammad et al. 2019; Molne, Verdrengh, and Tarkowski 2000; Verdrengh and Tarkowski 1997), while monocytes/macrophages play a detrimental role (Mohammad et al. 2019; Verdrengh et al. 2006).

3.2 Adaptive immunity

3.2.1 T- and B-cells

T-cells and B-cells constitute the major cell types involved in adaptive immunity. T-cells originate from the bone marrow, and migrate to the thymus where they mature and differentiate into several different subtypes among which CD8+, CD4+ and regulatory- T-cells are the most important ones. CD8+ cells, even referred to as cytotoxic T-cells are known for killing tumour cells as well as virus infected cells (Pennock et al. 2013; Rosendahl Huber et al. 2014). CD4+ T-cells, even known as T-helper cells, do not have killing capacity on their own but rather coordinate with and help other immune cells in eliminating the invading pathogen. CD4+ T-cells are in turn divided into several subtypes, such as Th1, Th2 and Th17 T-cells (Pennock et al. 2013; Zhu, Yamane, and Paul 2010). Regulatory T cells have the ability to supress immune responses of other cell types and have an important role in the maintenance of self-tolerance.

Recently, in a murine model, it was shown that CTLA4-Ig, a fusion protein that inhibits the second co-stimulatory signal required for activation of T-cells, significantly aggravated *S. aureus*-induced septic arthritis (Ali, Welin, et al. 2015). However, in another murine *S. aureus*-induced septic arthritis study, it was demonstrated that CD4+ T-cells aggravate disease severity since CD4+ T-cell depletion resulted in attenuated arthritis (Abdelnour et al. 1994). On the

other hand, mice in CD8+ T-cell depletion group exhibited similar outcomes as a control group, and CD8+ T-cells were thus shown to play a minor role in murine *S. aureus*-induced septic arthritis (Abdelnour et al. 1994).

In *S. aureus* Lpp-induced synovitis, our results revealed that T-cells play a minor role in the disease severity upon intra-articular injection of Lpl1 in the local murine knee model, as CD4+ and CD8+ T-cell depletion as well as CTLA4-Ig treatment gave rise to similar outcomes in the treated groups as in the control group (**Paper I**) (Mohammad et al. 2019). This suggests that T-cells in general were of minor importance in *S. aureus* Lpp-induced arthritis (Mohammad et al. 2019).

In *S. aureus*-induced skin infection model, a recent study showed that purified Lpp caused skin inflammation, accompanied with interferon γ producing T cell accumulation (Saito and Quadery 2018). CD4+ T-cells have been suggested to offer protection against secondary *S. aureus* skin and soft tissue infections (Montgomery et al. 2014).

B-cells constitute the other major arm of adaptive immunity. B-cells are known for secreting antibodies and thus play a key role in conferring humoral immunity. B-cells develop in the bone marrow and later migrate to the spleen for further maturation (Loder et al. 1999). In *S. aureus*-induced septic arthritis, B-cells play minor role compared to the other cell types, as mice deficient in B-cells displayed similar outcomes as their wild-type controls (Gjertsson et al. 2000). In the case of Lpp-induced joint inflammation, intra-articular injection of *S. aureus* Lpp did not trigger the influx of either B- or T-cells (**Paper I**) (Mohammad et al. 2019). In *S. aureus* skin infection, B-cells are known to produce antibodies that are directed against *S. aureus* virulence factors, and thus mediate important immune responses against the pathogen (Krishna and Miller 2012b).

3.3 Toll like receptors

Among the PRRs of the innate immune system are the TLR family, which plays a critical role in *S. aureus* recognition (Askarian et al. 2018). All TLRs possess a cytoplasmic Toll/interleukin–1 receptor (TIR) domain that enables initiation of intracellularly mediated signalling pathways (Takeda and Akira

2004). Several members of the TLRs contribute to the detection of conserved *S. aureus* molecules (Askarian et al. 2018), while TLR4 is a key PRR that detects lipopolysaccharide in Gram-negative bacteria (Takeuchi et al. 1999). TLR2 serves as a critical receptor for Lpp and also recognises synthetic lipopeptides (Aliprantis et al. 1999). It has been demonstrated that *S. aureus* Lpps, in fact, are considered as the most potent components in activating TLR2 (Hashimoto, Tawaratsumida, Kariya, Aoyama, et al. 2006; Hashimoto, Tawaratsumida, Kariya, Kiyohara, et al. 2006). TLR2 and its interaction with *S. aureus* Lpps are discussed in more detail below.

3.4 Chemokines and cytokines

Chemokines and cytokines play an essential role in cellular cross-talk during infectious and inflammatory conditions. Once secreted from the immune cells, they help to promote the recruitment and direct the activity of other immune cells in order to fight the infection/inflammation. While these molecules are a vital part of all inflammatory processes, pro-inflammatory cytokines can, when secreted in excessive levels, participate in aggravation of inflammatory and autoimmune diseases, such as rheumatoid arthritis (Feldmann, Brennan, and Maini 1996). Among the neutrophil chemoattractant chemokines are keratinocyte chemoattractant (KC) and macrophage inflammatory protein-2 (MIP-2) that originate from monocytes and macrophages (De Filippo et al. 2008). These are considered as the main recruiters of neutrophils and serve as ligands for the CXCR2 receptor (De Filippo et al. 2008; Lee et al. 1995). Furthermore, monocyte chemoattractant protein 1 (MCP-1) is a critical recruiter of monocytes/macrophages, while TNFα, IL-1, and IL-6, among others, are important pro-inflammatory cytokines that are involved in the upregulation of local and systemic inflammatory response.

The role and involvement of these chemokines and cytokines in murine *S. aureus*-induced infectious arthritis and skin infection are described briefly here.

IL-1 has been shown to be involved in the pathogenesis of septic arthritis (Ali, Na, et al. 2015; Hultgren, Svensson, and Tarkowski 2002). We recently showed that mice treated with IL-1 receptor antagonist (IL-1Ra), and infected with *S. aureus* had increased mortality and exhibited clinically and histologically more severe and frequent septic arthritis (Ali, Na, et al. 2015). We therefore assessed IL-1 importance in Lpl1-induced synovitis by treating

mice with IL-1Ra. Our results demonstrated that IL-1 did not play a major role in the induction of synovitis in this specific arthritis model (**Paper I**) (Mohammad et al. 2019).

The importance of TNF has also been studied in the context of staphylococcal infections. TNF has been shown to promote enhanced arthritis frequency in the haematogenous infection model while using a double deficient mouse strain, lacking TNF and lymphotoxin-alpha (Hultgren et al. 1998). Furthermore, Fei *et al*. previously demonstrated that anti-TNF therapy in combination with antibiotics led to favourable outcomes in murine septic arthritis (Fei et al. 2011). Intriguingly, anti-TNF treatment alleviated arthritis induced by antibiotic-killed *S. aureus* in local murine knee joints (Ali, Zhu, et al. 2015), further demonstrating a potent role of TNF in septic arthritis. Thus, we explored whether TNF inhibition had any beneficial effects in the Lpl1-induced synovitis by treating the mice with anti-TNF treatment (etanercept). TNF was indeed partially involved in modulating the arthritogenic effects in local *S. aureus* Lpl1-induced knee arthritis (**Paper I**) (Mohammad et al. 2019). Furthermore, inhibition of TNF was shown to reduce the skin lesions in mouse model of *S. aureus* skin infection (Na et al. 2017).

Decreased arthritis severity is closely associated with lower levels of IL-6 in local joints, suggesting that IL-6 is an important cytokine for maintenance of septic arthritis (**Paper I**) (Mohammad et al. 2019). We also demonstrated that *S. aureus* Lpps trigger the quick release of KC and MIP-2 in local tissues including knees and skin with enhanced influx of phagocytes, consequent inflammation and tissue damages (**Paper I** and **III**) (Mohammad et al. 2019).

3.5 Formation of *S. aureus* skin abscess and interplay with immune cells

Skin abscess lesions, not unique to *S. aureus* infections, are characterized as an infectious cavity filled with pus or translucent fluid within or below the skin surface, which may induce fluctuant swelling (Dryden 2009). Although most of the skin abscess lumps are self-limited and harmless, more serious cases are associated with a poorer outcome since *S. aureus* can disseminate within the blood and establish mature infections in virtually any of our internal organ systems (Kobayashi, Malachowa, and DeLeo 2015). Defects in the skin barrier, due to breaches or abrasions in the skin tissue, enable *S. aureus* to

penetrate into the damaged site and enter the underlying tissue. Once the bacterium has penetrated through the skin, resident immune cells, such as Langerhans cells and macrophages detect the pathogen and generate a rapid host inflammatory response at the local infection site (Miller and Cho 2011; Krishna and Miller 2012a). An interplay between the host immune system and the bacteria takes immediately place, and secretion of chemokines and cytokines is induced, which triggers the recruitment of phagocytes, specifically neutrophils (Miller and Cho 2011). As a result of the host's immune response, an abscess forms around the bacteria, known as a fibrous capsule, in order to try to eliminate as well as to limit the spread of *S. aureus* (Miller and Cho 2011). At the same time, *S. aureus* also possesses the ability to aide fibrin clots by using its component, Coa, as underscored earlier. The process of *S. aureus* skin abscess formation and the cutaneous immune response is depicted in Figure 2.

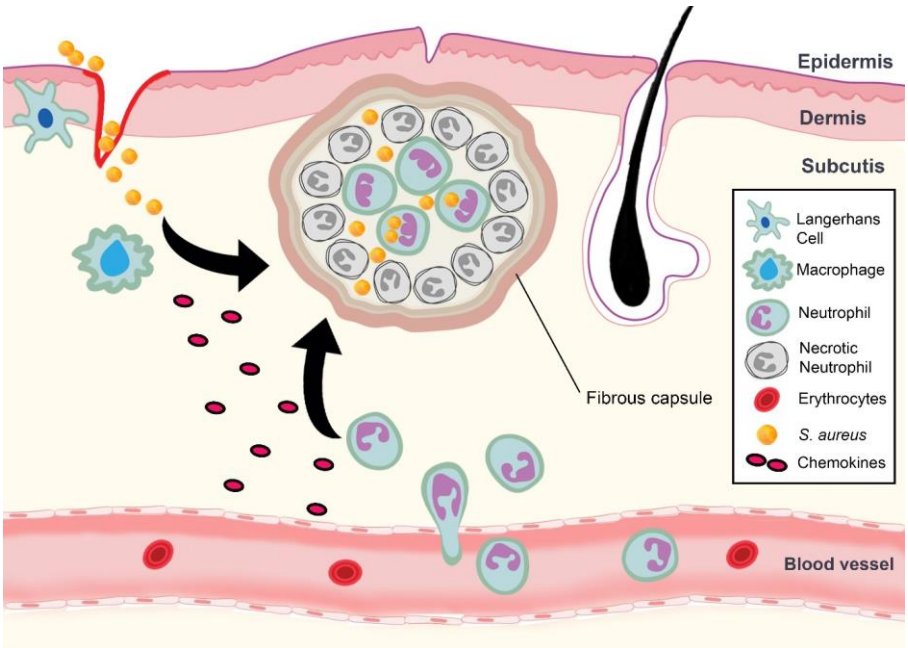


Figure 2. Illustration of Staphylococcus aureus skin abscess formation and cutaneous immune response.
Defects in the skin barrier enable S. aureus to enter the hosts´ underlying tissue. The invading pathogen interacts with resident immune cells which triggers the release of chemokines and pro-inflammatory cytokines followed by recruitment of neutrophils and abscess formation.

4. Bacterial lipoproteins

4.1 Biosynthetic pathway of lipoproteins

As an important part of the bacterial cell envelope homeostasis, lipidation of proteins naturally occurs as a posttranslational molecule reformation process, which ultimately forms mature Lpp in both Gram-positive and Gram-negative bacteria (Buddelmeijer 2015). Prior to Lpp maturation, precursor Lpp containing signal peptide with characteristic 'lipobox' undergoes a series of events in order to become lipidated. Initially, the precursor Lpps are transported into the cytoplasmic membrane through the general secretory (Sec) pathway, or in some cases via the twin arginine translocation (Tat) machinery, depending on the conformation of the protein (Hutchings et al. 2009). Pre-Lpp functions as precursor of Lpp and comprises an average of 16-27 amino acid long signal peptide sequence, resided at the N-terminal region, which is recognised by these protein-transporting machineries (Schmaler et al. 2010; Hayashi and Wu 1990). Importantly, this signal peptide also contains a specific motif at its C-terminal region, commonly known as the lipobox (Kovacs-Simon, Titball, and Michell 2011). The lipobox, characterized by a three-amino-acid sequence that is conserved and located in front of the indispensable cysteine residue (Nguyen and Gotz 2016), has an essential role in both guiding these proteins to the biosynthetic Lpp modification manufacturing site as well as in processing them into mature forms (Hutchings et al. 2009). Lpp modifications occur within the cytoplasmic membrane of the bacteria and are catalysed by three enzymes: 1) pre-Lpp diacylglyceryl transferase (*lgt*); 2) pro-Lpp signal peptidase (*lsp*); and 3) apo-Lpp *N*-acyltransferase (*lnt*) (Nakayama, Kurokawa, and Lee 2012; Sankaran and Wu 1994).

The first enzyme, *lgt* enables the transfer of a diacylglyceryl group from a phospholipid onto the thiol group located at the cysteine residue at the conserved lipobox motif (Schmaler et al. 2010; Nguyen and Gotz 2016; Tokunaga, Tokunaga, and Wu 1982; Sankaran and Wu 1994). Modification of the thiol group consequently allows membrane attachment and converts the **pre-Lpp** into a pro-Lpp (Kovacs-Simon, Titball, and Michell 2011). Followed by this lipidation, the **pro-Lpp** becomes recognizable by *lsp*, which subsequently cleaves the signal peptide at the N-terminal region (Hussain, Ichihara, and Mizushima 1982). This generates a newly formed amino-

terminal group at the cysteine residue that comprises a *S*-diacyl-glyceryl-cysteine residue and gives rise to the **apo-Lpp**, also known as the **diacylated Lpp**, which serves as a mature form of Lpp (Nakayama, Kurokawa, and Lee 2012). Finally, the *lnt* enzyme, which catalyses the third reaction, facilitates the transfer of another fatty acid derived from another phospholipid to the cysteine residue ultimately forming a **triacylated Lpp** structure (Nakayama, Kurokawa, and Lee 2012; Sankaran, Gupta, and Wu 1995). In contrast to the diacylated Lpp, the triacylated Lpp comprises an *N*-acyl-*S*-diacylated cysteine residue (Kurokawa, Ryu, et al. 2012). This biosynthetic pathway was initially described in Gram-negative *Escherichia coli* (Tokunaga, Tokunaga, and Wu 1982), and serves as the canonical biosynthetic pathway in Lpp (Nakayama, Kurokawa, and Lee 2012). *Lnt* is a necessity in most Gram-negative bacteria as the addition of the third fatty acid enables the Lpp to either maintain in the inner membrane or to release them to the outer membrane via a transporting pathway known as the localization of Lpp (Lol) system (Fukuda et al. 2002). However, the presence of the *lnt* homologues had so far only been demonstrated in certain Gram-positive bacteria with high-guanine-cytosine (GC) content, including mycobacteria (Tschumi et al. 2009; Brulle et al. 2010), streptomyces (Widdick et al. 2011), and *Corynebacterium* (Mohiman et al. 2012), while the typical *Escherichia coli lnt* homolog had still not been detected in low-GC content Gram-positive *Firmicutes* (Nakayama, Kurokawa, and Lee 2012). Therefore, it was supposed that *S. aureus* Lpps express a diacylated structure (Stoll et al. 2005). However, with the development of GC-MS technology, it has been shown that Lpp from Gram-positive bacteria may have different lipid structures (Kurokawa, Kim, et al. 2012), for example *S. aureus* containing tri-acylated Lpp. It indicates that the involvement of an unidentified *lnt* homolog does in fact exist in some low-GC content Gram-positive bacteria, such as *S. aureus* (Kurokawa et al. 2009; Asanuma et al. 2011; Navarre, Daefler, and Schneewind 1996; Shahmirzadi, Nguyen, and Gotz 2016; Stoll et al. 2005) and *Bacillus subtilis* (Hayashi et al. 1985).

Intriguingly, it was later revealed that *S. aureus*, in fact, does possess a triacylated lipid-moiety (Kurokawa et al. 2009). However, the lack of *lnt* homolog still raised the enigmatic question of how *S. aureus* is capable of forming triacylated mature forms of Lpp. Evidence points toward that *S. aureus* bears another type of acyltransferase, and this enzyme serves as a prerequisite in ultimately yielding the triacylated lipid-moiety structure of the

Lpp (Kurokawa et al. 2009). Furthermore, Asanuma *et al*. provided structural evidence of *N*-acylation of the lipid-moiety through α-aminoacylation at the lipidated cysteine residue in *S. aureus*, which enabled the addition of a third and last fatty acid that ultimately gave rise to the triacylated *S. aureus* Lpp structure (Asanuma et al. 2011). In addition, triacyl-Lpp structures were detected in several *S. aureus* strains as well as in *S. epidermidis* (Asanuma et al. 2011). However, certain factors, such as bacterial growth phase and acidic pH, have been shown to alter the conversion from di- to triacyl-Lpp in *S. aureus* (Kurokawa, Kim, et al. 2012). Prior to this study, it was widely believed that in each bacterium, bacterial Lpps were consistent in forming one lipidated structure exclusively, independently of the influence of the environmental conditions (Nakayama, Kurokawa, and Lee 2012). As a result of the important finding demonstrated by Kurakowa *et al.* (Kurokawa, Kim, et al. 2012), the concept that each bacterium is biosynthetically processed in an unperturbed manner ultimately leading to either a diacylated or triacylated Lpp, has thus changed. Potential reasons for these structural Lpp alterations in *S. aureus* might be due to a possible down-regulation of the specific enzyme that facilitates the addition of a third fatty acid with regard to its activity or specificity during specific environmental conditions that consequently may inhibit its catalysing functions.

Previous study identified a conserved Lpp structure containing an *N*-acyl-*S*-monoacyl-glyceryl-cysteine, termed the lyso form, which exists in several low-GC Gram-positive bacteria, including *Bacillus cereus*, *Enterococcus faecalis*, *Streptococcus sanguinis*, and *Lactobacillus bulgaricus* (Kurokawa, Ryu, et al. 2012). The mechanism behind the lyso structure formation was unknown until recently as Armbruster *et al*. successfully identified the gene product that facilitates this particular reaction, commonly known as the Lpp intramolecular transferase (*lit*) enzyme, which distinctly differs from the canonical *lnt* enzyme that plays an essential role in forming the triacylated Lpp structure in Gram-negative bacteria (Armbruster and Meredith 2017). The authors propose that the *lit* enzyme permits the transfer of a fatty acid to the lipidated cysteine by utilizing an internal transferring mechanism that reallocates one of the fatty acids from the diacylglycerol moiety to the α-amino group in low-GC Gram-positive bacteria, including *Enterococcus faecalis* and *Bacillus cereus* (Armbruster and Meredith 2017). In fact, it was very recently reported that the *lit* enzyme facilitated this intramolecular transfer process through involvement

of acyl chain migration (Armbruster, Komazin, and Meredith 2020). Furthermore, in *Listeria monocytogenes*, it was recently demonstrated that the *lit* ortholog, *lit*2, has a copper-induced expression effect, which consequently transforms the lipid-moiety structure as it shifts from being diacylated to form a lyso Lpp structure (Armbruster, Komazin, and Meredith 2019).

Importantly, in a very recent study, it was revealed that a two-component system, denoted the Lpp *N*-acylation transferase system (*lns*), was responsible for the *N*-acylated triacyl-Lpp form in *S. aureus* (Gardiner et al. 2020), which distinguishably differs from the *lnt* enzyme that is implemented in Gram-negative bacteria (Gupta and Wu 1991; Robichon, Vidal-Ingigliardi, and Pugsley 2005; Wiktor et al. 2017). The two genes, *lnsA* and *lnsB*, were found to be exclusively presented in staphylococcal species that are currently known to possess a triacylated Lpp lipid-moiety and did not exhibit any similarities with neither the *lnt* nor the *lit* bacterial Lpp *N*-acylating enzymes (Gardiner et al. 2020). The pivotal role of *lnsAB* in yielding the triacylated Lpp structure in *S. aureus* was further strengthened by the fact that a disruption of this two-gene system reverted the *S. aureus* triacylated Lpp into diacylated Lpp (Gardiner et al. 2020). This important finding regarding *S. aureus* Lpp conversion might actually explain how *S. aureus* is able to form di- or triacyl-Lpp structures depending on the environmental factors as Kurokawa and colleagues reported earlier (Kurokawa, Kim, et al. 2012), and also suggest that *lnsA* and/or *lnsB* are sensitive to such environmental parameters. Gardiner *et al*. further showed that while integrating *lnsA* and *lnsB* into the genome of *Listeria monocytogenes*, the Lpp structure changes from initially being diacylated to becoming triacylated (Gardiner et al. 2020). The interesting finding of this new staphylococcal Lpp tailored machinery will most likely initiate more studies in the near future that hopefully will provide a more detailed insight of their functions. The biosynthetic pathway of bacterial Lpps are illustrated in Figure 3.

Of note, not only variation with regard to the lipidation with di- or triacylated forms of Lpp as a final bacterial molecular structure are found in Lpp, but also the fatty acids, originating from membrane phospholipids and shaping the structure of the lipid-moiety, vary in length among different kind of bacteria (Buddelmeijer 2015). Nevertheless, in *S. aureus*, a long-chain fatty acid is characteristically presented at the *N*-acyl position of the lipid-moiety in the triacylated form of the Lpp (Kurokawa et al. 2009; Nguyen et al. 2017).

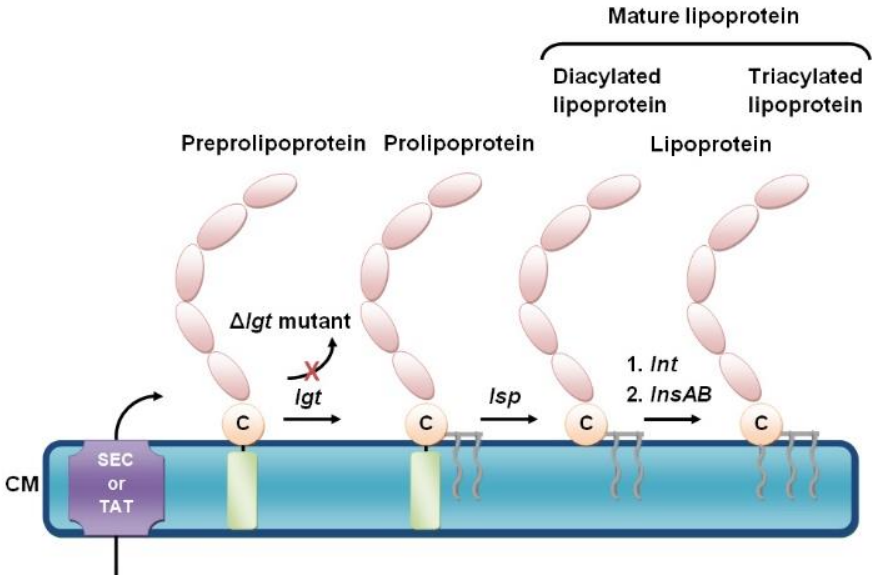


Figure 3. The biosynthetic pathway of bacterial lipoproteins.
Lipoproteins are synthesised as preprolipoprotein precursors, which comprise an N-terminal signal peptide sequence (depicted as light-green cylinder), and are translocated across the cytoplasmic membrane (CM) by either the general secretory (Sec) or twin arginine translocation (TAT) pathways. The first enzyme, preprolipoprotein diacylglyceryl transferase (lgt) enables the transfer of a diacylglyceryl moiety to the indispensable cysteine residue (depicted as a beige circle with the letter, C), which forms a prolipoprotein. This lipid modification is followed by the second enzyme, prolipoprotein signal peptidase (lsp) that cleaves the signal peptide and generates a mature diacylated lipoprotein. For some bacterial lipoproteins, a third enzyme is required in order to form a mature triacylated lipoprotein. This lipid acylation is catalysed by either 1) apolipoprotein N-acyltransferase (lnt) and that occurs in most Gram-negative bacteria, or 2) lipoprotein N-acylation transferase system (lnsAB) and that was recently demonstrated to occur in Gram-positive Staphylococcus aureus. By mutating the catalytic lgt enzyme (Δlgt mutant), the maturation of lipoproteins is inhibited and can thus no longer be lipidated.

The importance of bacterial Lpp can be studied in numerous ways. Firstly, by inhibiting the maturation of the Lpp by mutating the specific catalytic enzymes, *lgt* and *lsp*, referred to as Δ*lgt* and Δ*lsp* deletion mutants, respectively. The Δ*lgt* mutant leaves the cysteine residue unmodified, hence preventing lipidation to occur (Nguyen and Gotz 2016). However, as the signal peptide of pre-Lpp directs the immature form of the Lpp to the cytoplasmic membrane, as previously mentioned, the pre-Lpp still forms an initial linkage to the membrane (Nguyen and Gotz 2016). This procedure also prevents the *lsp* enzyme to fulfil its role due to the lack of pro-Lpp recognition (Nguyen and Gotz 2016). Regarding the Δ*lsp* mutant, the first lipid-modification of the Lpp is initiated, however, the signal peptide remains intact rather than cleaved (Nguyen and Gotz 2016). This ultimately leads to a disturbed balance within the Lpp biosynthetic machinery and may result in improper accumulation of immature Lpp.

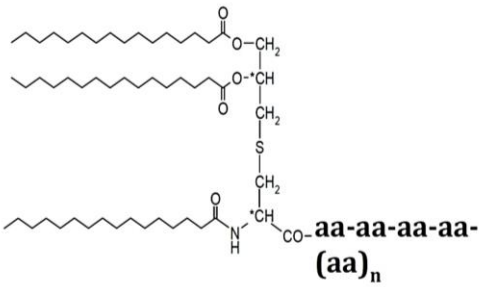
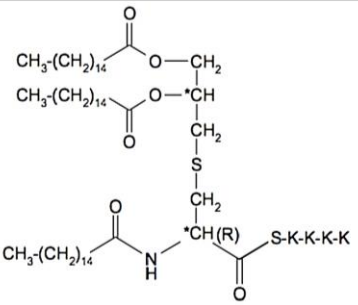
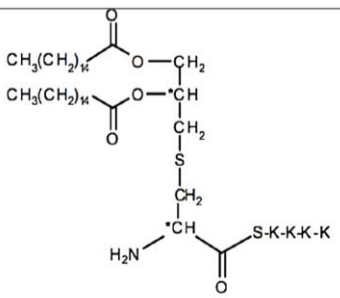
Secondly, the significance of bacterial Lpp can also be investigated by isolation and purification of specific Lpp from the bacteria of interest, or thirdly, by using synthetic lipopeptides that resemble the lipid-moiety structure of bacterial Lpp, such as Pam3CSK4 (triacylated lipid form) or Pam2CSK4 (diacylated lipid form).

S. aureus Δ*lgt* mutant strains have been implemented in all studies within my thesis (**Paper I-III**), whereas purified *S. aureus* Lpp Lpl1 along with the synthetic lipopeptides Pam3CSK4 and Pam2CSK4 have been used in **Paper I and Paper III**.

The outcomes of bacterial deletion mutants as well as purified Lpp are briefly reviewed below.

Table 1 is a list with structures of the compounds used in this thesis.

Table 1. The list of compounds used in this thesis.

Compounds	Structures	Molecular weight (g/mol)
Lpl1(+sp) from SA113		≈ 29210
Lpl1(-sp) From SA113 Δ*lgt* mutant	**aa-aa-aa-aa-(aa)$_n$**	28350
Pam3CSK4		1509
Pam2CSK4		1271

Adapted with minor modifications from Frontiers in Cellular and Infection Microbiology (Nguyen et al. 2016).

4.2 Types and functions of *S. aureus* lipoproteins

S. aureus typically expresses Lpp in approximately 2-3% of its total bacterial genome (Babu et al. 2006). *S. aureus* Lpps can be grouped into different functional categories, whereby most of their functions appear to be achieved between the border of the bacterial cell wall and the membrane (Schmaler et al. 2010; Hutchings et al. 2009).

Lpps represent a major class of surface proteins in *S. aureus* (Nguyen and Gotz 2016). The role of Lpp in staphylococci involves various surface associated functions, such as nutritional acquisition as well as respiration, protein secretion, and invasion (Shahmirzadi, Nguyen, and Gotz 2016; Graf et al. 2018). Other functions comprise synthesis of the bacterial cell wall, transmission of cell signalling, transport of electrons, and surface stress signalling response mechanisms (Graf et al. 2018). Lpps also participate in antibiotic resistance (Sutcliffe and Russell 1995). Hence, all these diverse functions are in principal for the bacterial cell envelope and its survival.

Lpp is characteristically divided into two functional entities, whereby the protein-moiety serves and maintains the bacteria with its metabolic nutrition and function, whereas the lipid-moiety has a key role in anchoring the protein into the bacterial membrane as well as playing an important role in pathogenicity (Nguyen et al. 2017; Shahmirzadi, Nguyen, and Gotz 2016). As the lipid-moiety of *S. aureus* Lpp is situated at the outer leaflet of the cytoplasmic membrane, its triacylated fatty acid structure is incorporated into the membrane, while the protein-portion is directed towards the cell wall and beyond (Nguyen and Gotz 2016; Shahmirzadi, Nguyen, and Gotz 2016).

To date, up to 70 Lpps have been detected in *S. aureus*, and the number of Lpps vary within various *S. aureus* genomes (Nguyen and Gotz 2016). By utilizing a Lpp predicting tool called ParSeq (Schmollinger et al. 2004), it was previously revealed that 55 putative Lpps, with the expression of a consisting signal peptide of regular length, exist in the genome of *S. aureus* N315 (Stoll et al. 2005). Many of those Lpps that exhibited similarities to proteins with notorious function were elucidated as transporters for iron, zinc, sugar, amino acid, teichoic acid, and oligopeptide among others, while other Lpps were

associated to various functions of enzymes, such as phage terminases, protein-disulfide isomerase, and pyruvate-formate-lyase-activating enzyme, along with an additional set of other enzymatic functionalities (Stoll et al. 2005). On the other hand, a recent study re-evaluated Lpp more specifically in *S. aureus* USA300 strain (Shahmirzadi, Nguyen, and Gotz 2016), a pathogenic strain well-known for its multiple antibiotic resistance that serves as the predominant source of community-associated infections (Diep et al. 2006). This study proposed that *S. aureus* USA300 strain devotes 2.6% of its total gene coding ability possessing around 67 Lpps, an identification that was based on the PRED-LIPO prediction program (Shahmirzadi, Nguyen, and Gotz 2016). Nearly half of the total proportion were involved in transport of ions and nutrients, amino acids, peptides, enzymes and chaperons; 22% were classified as tandem Lpps including a specific set referred to as lipoprotein-like (*lpl*) genes; while 28% were of unknown function (Shahmirzadi, Nguyen, and Gotz 2016). Of note, eight (12%) of the 67 identified Lpps were annotated as iron transporters (Shahmirzadi, Nguyen, and Gotz 2016), a relatively high proportion of Lpps specially designed to provide the bacteria with uptake of the needed iron. This is of essential importance during infectious conditions as iron is known to serve as a key element for the survival of the bacterium (Braun 2001). As iron and nutrient transport alone accounts for 36% of the total function of *S. aureus* Lpp in this model strain (Shahmirzadi, Nguyen, and Gotz 2016), one of the major roles of Lpps is to maintain and improve the metabolic activity and fitness of the bacterium.

Another study inspecting the genome of a large set of complete genomic sequences of *S. aureus* strains, more precisely 123 genome sequences, recently revealed that an average number of 70 Lpps was estimated per strain, which equals to 2.6% of the bacterial genome (Graf et al. 2018). In addition, approximately 30% of all these *S. aureus* Lpps were annotated as importers of substrate-binding proteins through the ATP-binding cassette (ABC) transport system (Graf et al. 2018). Overall, this is in very close resemblance with the predicted Lpp number reported for the *S. aureus* USA300 strain (Shahmirzadi, Nguyen, and Gotz 2016). Since a large set of *S. aureus* Lpps has still not been characterized, the need to elucidate their function is fully warranted.

Many pathogenic *S. aureus* strains harbour a genomic island, termed νSaα, which is designated as non-phage and non-staphylococcal cassette chromosome (Baba et al. 2008; Diep et al. 2006; Kuroda et al. 2001).

Furthermore, the νSaα-island is incorporated at certain chromosomal loci that are unique for the *S. aureus* genome (Tsuru and Kobayashi 2008). This genomic island possesses highly conserved genes, which comprise two clusters of tandem repeat sequences; one encodes exotoxin genes, whereas the other cluster encodes a number of homologous Lpps, termed lipoprotein-like (*lpl*) genes (Nguyen et al. 2015; Baba et al. 2008), all of which contain the conserved lipobox motif (Babu et al. 2006). The largest *lpl* gene cluster is preserved at the νSaα islet but additional clusters also occur at other chromosomal locations within the *S. aureus* genome, approaching to up to five *lpl* gene clusters per genome (Graf et al. 2018). The *lpl* genes are typically aligned in tandem paralogous clusters and share significant homologous properties when they originate from the same *lpl* cluster (Tsuru and Kobayashi 2008). In the USA300 strain, nine *lpl* gene homologs are encoded in the genomic island (Nguyen et al. 2015; Shahmirzadi, Nguyen, and Gotz 2016). The first *lpl* gene product from the νSaα-specific *lpl* cluster, named Lpl1, has been extensively utilised in this thesis (**Paper I and Paper III**). Specifically, I have studied the effect of purified *S. aureus* Lpp, with or without the signal peptide, i.e. Lpp expressing the lipid-moiety, denoted Lpl1(+sp), or Lpp lacking the lipid-moiety, denoted Lpl1(-sp) in an animal model of skin and joint inflammation.

As highlighted above, there are various types of Lpps in *S. aureus* with diverse functions. However, there is also a high variation with regard to their sizes as they range between 6-89 kDa in the *S. aureus* USA300 strain, with an average size designated between 30-50 kDa (Shahmirzadi, Nguyen, and Gotz 2016).

5. Lipoproteins and host immune response

5.1 *S. aureus* Lpp – a potent immune stimulator

It was widely believed that LTA and PGN, important cell wall components of *S. aureus*, are the main immune stimulators (Fournier and Philpott 2005; Takeuchi et al. 1999; Nguyen and Gotz 2016). However, recent studies suggest that the inflammation caused by LTA or PGN is more likely due to contamination with Lpp (Hashimoto, Tawaratsumida, Kariya, Kiyohara, et al. 2006; Hashimoto, Tawaratsumida, Kariya, Aoyama, et al. 2006; Zahringer et al. 2008). This was proven in an experiment showing that a *S. aureus* Δ*lgt* mutant strain displayed diminished innate immune activation in contrast to its parental Lpp-expressing strain (Stoll et al. 2005). Furthermore, using LTA from a *S. aureus* Δ*lgt* strain, thus excluding any risks of Lpp contamination, dramatically decreased the innate immune response when compared to its parental strain, even though the structures of the LTA components were identical in both strains (Hashimoto, Tawaratsumida, Kariya, Kiyohara, et al. 2006). Suspicions arose whether previous conclusions regarding LTA and PGN interaction with certain PRRs were due to Lpp contamination. In fact, several reports have indicated that *S. aureus* PGN, purified from the *S. aureus* Δ*lgt* strain, lacked the ability to activate TLR2 (Volz et al. 2010; Schaffler et al. 2014). PGN was shown to co-localise with both nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and TLR2 after internalization in mouse keratinocytes (Muller-Anstett et al. 2010). Overall, the theory that the innate immune sensation of *S. aureus* PGN or LTA operated exclusively through TLR2 was thus more likely a result of Lpp contamination, which further indicates the importance of *S. aureus* Lpp in immune activation via TLR2.

In this thesis, we have utilised and explored the importance of *S. aureus* Lpp in our well-established murine *S. aureus*-induced septic arthritis models via two putative routes of infections, one of which is a local route of infection (**Paper I**), and a second one is a systemic route of infection (**Paper II**). In addition, the role of *S. aureus* Lpp in a murine skin inflammation and infection model was also investigated (**Paper III**).

5.2 *S. aureus* Lpp – a predominant TLR2 agonist

Lpp maturation plays an essential role in immune signalling, inflammation, and pathogenicity during *S. aureus* infections (Stoll et al. 2005; Nguyen and Gotz 2016; Nguyen et al. 2015). The lipid structures of Lpp in *S. aureus* are known as MAMPs (Nguyen and Gotz 2016), being vital activators of the innate immunity as they play a potent role in alerting PRRs in host cells (Nguyen et al. 2017). Despite the fact that the lipid-moiety is embedded in the membrane, fractions from a minor proportion of mature Lpp in *S. aureus* tend to be released from its origin, enter the cell wall, and parts of the lipidated structures can be exposed on the cell surface (Stoll et al. 2005). The lipid modification is an absolute necessity in activating the host immune signalling as Lpp, that lacks the lipid structure, displays no such stimulatory activity (Stoll et al. 2005; Nguyen and Gotz 2016). Thus, the lipid-moiety functions as an important danger signal towards the host (Nguyen and Gotz 2016; Nguyen et al. 2017). Lpp and/or lipopeptides are recognised as the predominant ligands for TLR2 (Hashimoto, Tawaratsumida, Kariya, Aoyama, et al. 2006; Aliprantis et al. 1999; Brightbill et al. 1999). In fact, the unique thioether linkage that comprises a diacylglycerol moiety, which is commonly represented in all bacterial Lpps, serves as the core pattern for ligand recognition by TLR2 (Nakayama, Kurokawa, and Lee 2012). TLR2 facilitates a broader range of ligand sensing due to heterodimerization with various co-receptors (Fournier 2012; Lee, Avalos, and Ploegh 2012). In fact, this is the case during Lpp/lipopeptide recognition as TLR2 forms heterodimers with the additional receptors, TLR1 or TLR6 (Fournier 2012). Depending on the lipid structure of the Lpp, or the structure of synthetic lipopeptides, activation of the following TLR2 heterodimers occur: TLR2/TLR1 heterodimers sense triacylated Lpps and Pam3CSK4 lipopeptide (Jin et al. 2007), while TLR2/TLR6 heterodimers sense diacylated Lpps and Pam2CSK4 lipopeptide (Takeda, Takeuchi, and Akira 2002; Takeuchi et al. 2002; Kang et al. 2009). Thus, formation of such dimeric complexes through TLR2 with other TLRs is an important feature for the host immune cells in the recognition of distinct Lpp/lipopeptide structures (Takeda, Takeuchi, and Akira 2002).

As TLR2 possesses this heterodimerisation ability, formation of the TLR2/TLR1 or TLR2/TLR6 dimerization complexes ultimately generates distinctive binding pockets, which enable incorporation and detection of the

different lipid structures found in bacterial Lpps and lipopeptides (Schenk, Belisle, and Modlin 2009). Upon interaction of TLR2 with Lpps/lipopeptides and the initiation of TLR2 dimerization, the two ester-bound fatty acid chains, i.e. the diacylglycerol moiety found in the thioether linkage of the cysteine residue in Lpps, bind to a pocket in TLR2 regardless of whether Lpps/lipopeptides possess a two- or three lipid chain (Jin et al. 2007; Schenk, Belisle, and Modlin 2009). In terms of the three-lipid chain (triacylated Lpp or Pam3CSK4), the amide-linked fatty acid binds to a hydrophobic pocket in TLR1 (Jin et al. 2007; Schenk, Belisle, and Modlin 2009). However, in the case of the two-lipid chain that comprises of a free α-amino-terminal (diacylated Lpp or Pam2CSK4), no such accommodation occurs, and heterodimerisation with TLR6 is facilitated instead (Kang et al. 2009). As the TLR1 pocket utilises the space to bind the *N*-acyl chain (Jin et al. 2007), the pocket in TLR6, on the other hand, is considerably smaller, has lower affinity towards the three-lipid chain, and is further blocked due to the phenylalanines being present (Kang et al. 2009). Overall, this shows that TLR2 interacts with bacterial Lpps/lipopeptides differently depending on their lipid chain structure, although the activation of both of these TLR complexes mediates the same signalling pathway (Schenk, Belisle, and Modlin 2009). The N-terminal cysteine modification in bacterial Lpp (diacylated or triacylated structures) ultimately commands which type of TLR2 complexes (TLR1 or TLR6) that will form and lead to Lpp-TLR2 interaction. The recognition of these *S. aureus* components elicits innate immune signalling that further modulates the host's immune response and the inflammatory reactions (Nguyen and Gotz 2016).

Upon Lpp-TLR2 interaction, an intracellular response is initiated, and in turn yields a cascade of events that comprise numerous phosphorylation steps ultimately culminating in NF-κB (nuclear factor kappa B) activation (Takeda and Akira 2004; Norgard et al. 1996; Rawadi et al. 1999). This signalling transduction pathway is mediated through the TIR domain-containing adaptor protein, MyD88 (myeloid differentiation primary response gene 88) (Takeda and Akira 2004). As MyD88 interacts with the cytoplasmic TIR domain of the TLR (Takeda and Akira 2004), a downstream signalling pathway is facilitated, which involves the recruitment of the kinases, IL-1 receptor-associated kinase (IRAK)-4 and IRAK-1 (Takeda and Akira 2004; Lin, Lo, and Wu 2010) that form a complex, termed the Myddosome (Lin, Lo, and Wu 2010). In turn, this signalling-mediated complex participates in the activation and interaction with the protein TRAF6 (tumour necrosis factor receptor–associated factor 6)

(Takeda and Akira 2004; Barton and Medzhitov 2003). The Myddosome complex mediates further phosphorylation that is associated with TRAF6 and leads to the activation of TAK1 (transforming growth factor β-activated kinase 1) and the inhibitor of NF-κB kinase (IKK) complex (Sato et al. 2005). Subsequently, this activates the NF-κB and permits the release of various cytokines and chemokines (Muzio et al. 1998; Sato et al. 2005). The pathway is illustrated in Figure 4.

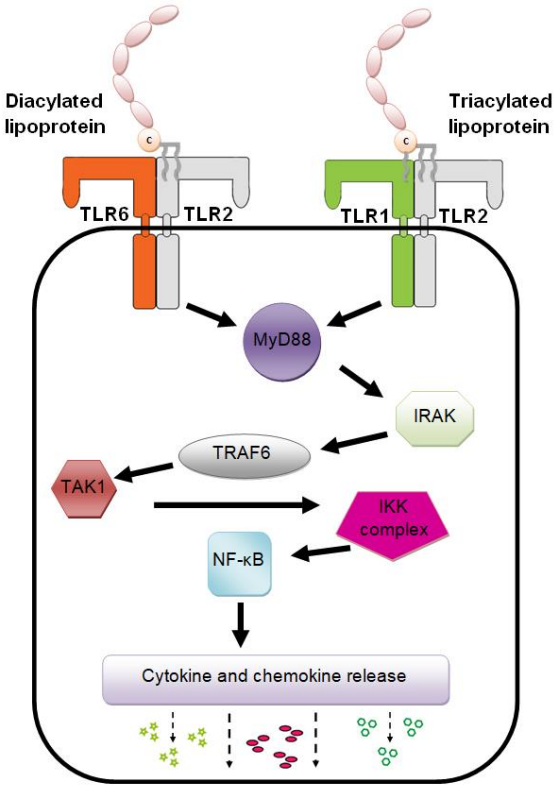


Figure 4. An overview of the TLR signalling pathway triggered by lipoproteins.
Binding of diacylated or triacylated lipoprotein on TLR2/6 and TLR2/1, respectively, triggers a cascade of events that culminate in the release of pro-inflammatory cytokines and chemokines. MyD88 = myeloid differentiation primary response gene 88, IRAK = interleukin-1 receptor-associated kinase, TRAF6 = tumour necrosis factor receptor–associated factor 6, TAK1 = transforming growth factor β-activated kinase 1, IKK = inhibitor of NF-κB, NF-κB = nuclear factor kappa B.

Importantly, the construction of *S. aureus* Δ*lgt* mutants along with improved methods for extraction and purification of Lpps (Stoll et al. 2005), have evidently proved that TLR2 is a major receptor exclusively activated by Lpps/lipopeptides (Bubeck Wardenburg, Williams, and Missiakas 2006; Hashimoto, Tawaratsumida, Kariya, Aoyama, et al. 2006; Hashimoto, Tawaratsumida, Kariya, Kiyohara, et al. 2006). Of note, purified manganese transport protein C (MntC) or staphylococcal iron transport protein C (SitC) serve as the first native *S. aureus* Lpp molecules to potently stimulate and to co-localise with TLR2 (Kurokawa et al. 2009; Muller et al. 2010). However, the mechanism behind the Lpp release in *S. aureus*, and how the immune system actually facilitates the detection of Lpp remained elusive until very recently. It was suggested that the coordinated action of PSM peptides plays a role here (Hanzelmann et al. 2016; Schlatterer et al. 2018). Hanzelmann *et al*. demonstrated that these peptides enable *S. aureus* to release Lpp from their otherwise trapped cytoplasmic membrane lipid-linkage, and subsequently promote Lpp mobilization, a feature that may only occur in the presence of PSMs (Hanzelmann et al. 2016). Schlatterer *et al*. revealed that PSMs prompt the release of Lpp as vesicle components in *S. aureus* (Schlatterer et al. 2018). PSMs are therefore a necessity to present *S. aureus* Lpp to TLR2 and provoke its activation (Hanzelmann et al. 2016; Schlatterer et al. 2018). Intriguingly, acquisition of the *lnsAB* complex, which yields the triacylated form of Lpp in *S. aureus*, subsequently facilitates the transition of TLR2/TLR6 heterodimer to form a TLR2/TLR1 heterodimer instead (Gardiner et al. 2020). This suggests that the Lpp catalysing acyl transferase machinery along with *lnsAB* dictates which partner TLR2 will form heterodimer-complexes with.

The addition of a third fatty acid at the amino group of the cysteine residue, which gives rise to a triacylated lipid structure, has a critical function for substrate recognition and the release of Lpps with efficient transport towards the outer membrane of Gram-negative bacteria through the Lol system (Fukuda et al. 2002). However, since Gram-positive bacteria lack an outer membrane (Silhavy, Kahne, and Walker 2010), the transporting system conducted by the Lol apparatus is not utilised. This indicates that the amide-linked fatty acid at the N terminus in Gram-positive bacteria (Hutchings et al. 2009) fulfils other functions beyond the involvement of Lol-mediated transport.

As the *lit* enzyme forms the lyso Lpp structure, possessing two acyl chains at the lipid-moiety (Armbruster and Meredith 2017), a resulting lyso structure was shown to preferentially mediate signalling via the TLR2/TLR6 complex despite the conduction of *N*-acylation (Armbruster, Komazin, and Meredith 2019). This lyso Lpp structure was reported to exhibit attenuated TLR2 recognition in contrast to the conventional di- or triacyled Lpp structures (Armbruster, Komazin, and Meredith 2019), which suggests that various modifications or remodelling of the N-terminal end leads to altered TLR2 recognition. As a consequence, this might help the bacteria to evade immune recognition (Armbruster, Komazin, and Meredith 2019). Moreover, this further proves the concept that different lipid-moiety structures, including the lyso form, influence the TLR2-stimulating effect, since various Lpp modifications seem to function as either more or less dominant TLR2 agonists (Armbruster, Komazin, and Meredith 2019; Gardiner et al. 2020). The TLR2 sensing ability varies considerably between different bacterial species (Nguyen and Gotz 2016). Nevertheless, among the immense range of virulence factors staphylococci dispose, Lpps are still considered as the main immunobiologically active components (Hashimoto, Tawaratsumida, Kariya, Aoyama, et al. 2006; Hashimoto, Tawaratsumida, Kariya, Kiyohara, et al. 2006; Nguyen and Gotz 2016).

It may seem paradoxical in bacterial evolution that Lpp evokes a cell-mediated immune response, specifically through TLR2-MyD88 signalling, thus initiating a battle between the *S. aureus* Lpp and the host immune system. However, this skilful bacterium is known to utilise various functions in order to escape immune recognition (Foster et al. 2014). A good example is staphylococcal superantigen-like protein 3 (SSL3), which is a TLR2 antagonist (Koymans et al. 2015). SSL3 prevents TLR2 dimerization with its co-receptors by forming a complex that partially closes off the pocket in the TLR2, which consequently inhibits the binding between Lpp and TLR2 (Koymans et al. 2015).

5.3 Importance of Lpps in iron acquisition and metabolic fitness

As Lpps dispose a vast range of important functionalities in bacteria, lipidation of bacterial Lpps has been strongly associated with virulence (Nguyen and

Gotz 2016). In fact, Lpp maturation serves as a necessity in many bacterial species in their battle against the host (Kovacs-Simon, Titball, and Michell 2011). Investigation of pathogenic properties induced by Lpp has mostly been conducted through implementation of *lgt* or *lsp* mutants in various *in vitro* and *in vivo* settings. Important parameters, such as the ability to acquire ions and nutrients as well as bacterial survival and bacterial proliferation have thus been studied in a broader extent. Lpp maturation has been shown to be of fundamental importance in iron acquisition, stimulating *S. aureus* bacterial growth, as *S. aureus* SA113 Δ*lgt* mutant strain exhibited growth defects compared to its parental strain under nutrient-restricted conditions (Stoll et al. 2005). In line with these results, we demonstrated that the Δ*lgt* mutant had impaired growth compared with its parental strain in nutrient-poor conditions, while the Δ*lgt* mutant and parental strain had similar growth rates in nutrient-rich conditions (**Paper II**) (Mohammad et al. 2020). To our surprise, we also found in a co-cultivation assay that *S. aureus* Newman parental strain competed out the Δ*lgt* mutant strain even in nutrient-rich conditions, although only during the early time-points (**Paper II**) (Mohammad et al. 2020), suggesting that Lpp expression promotes a competitive behaviour, which may be advantageous for the bacteria. Another study further underlined the importance of maturation of Lpp as a prerequisite for the bacteria in order to grow as well as to acquire iron both *in vitro* and *ex vivo* (Schmaler et al. 2009). Schmaler *et al*. demonstrated that while first starving the bacteria in an iron-depleted environment, followed by supplementation with iron-sources, hence restoring the iron availability, SA113 Δ*lgt* mutant strain exhibited impaired iron dependent bacterial growth compared to its parental strain (Schmaler et al. 2009). The concept of 'better iron acquisition, better bacterial growth' was further verified in an assay using streptonigrin, which is an iron-dependent antibiotic that craves iron in order to fulfil its bactericidal effect (Yeowell and White 1982). Δ*lgt* displayed both less streptonigrin sensitivity and reduced accumulation of iron (Schmaler et al. 2009). Likewise, it was demonstrated that Lpp expression influenced the acquisition of iron during systemic infection as *ex vivo* assessment of mouse kidneys exposed to the SA113 parental strain were more sensitive to streptonigrin than those exposed to SA113 Δ*lgt* mutant strain (Schmaler et al. 2009). Altogether, these findings strongly imply the importance of Lpp maturation in iron uptake in *S. aureus*. In addition, Bubeck Wardenburg *et al*. demonstrated that the growth of Newman Δ*lgt* mutant strain was retarded in contrast to its parental strain in

human whole blood and activated macrophages, although the proliferation rate was similar between the two strains in human sera (Bubeck Wardenburg, Williams, and Missiakas 2006).

The expression of genes associated with Lpp and iron acquisition is known to be changed during iron deprivation (Allard et al. 2006), and expression of various staphylococcal Lpps that participate in the iron uptake has been shown to be greatly upregulated in such conditions (Morrissey et al. 2000; Heinrichs et al. 1999; Sebulsky and Heinrichs 2001; Dale, Sebulsky, and Heinrichs 2004; Hempel et al. 2011). Acquisition of iron is a pervasive feature employed by pathogens, such as *S. aureus*, in order to improve their survival and proliferation (Hammer and Skaar 2011; Haley and Skaar 2012). These evolving mechanisms in staphylococci have widely been attributed to be a part of their success during host-pathogen interactions (Sheldon and Heinrichs 2012).

Overall, Lpp in *S. aureus* plays an essential role in enabling the bacteria to acquire sufficient loads of iron under infectious conditions. As free iron ions are limited in the host environment and the iron supply is of critical importance for the survival of the staphylococcal pathogen (Sheldon and Heinrichs 2012; Hammer and Skaar 2011), inhibition of the Lpp function, through incomplete Lpp maturation, can be deleterious to the metabolic fitness of the bacterium, consequently prompting it to cease in the battle against the host.

The largest iron reservoir in the host is represented by the heme iron, which serves as the preferred iron source for *S. aureus* (Skaar et al. 2004). As *S. aureus* seeks ways to acquire sufficient iron uptake during an infection, the host innate immune system employs defensive mechanisms by limiting the iron availability for the invading bacteria, functioning as one of the primary host defence responses during infection (Cassat and Skaar 2013; Haley and Skaar 2012). *S. aureus* can therefore utilise Lpp as an efficient iron transporter in order to circumvent this nutritional immunity, increase its chances of survival and consequently cause a disease. Therefore, maturation of Lpp plays a potent role during infection as it possesses the ability to boost the bacteria with the needed iron- and nutrient supply. The loss or disturbances of iron-regulated systems during *S. aureus* infections are strongly associated with attenuated virulence (Torres et al. 2006; Pishchany et al. 2014), which further

implies the critical role iron serves in the pathogenesis of diseases this bacterium causes.

In other Gram-positive bacteria, such as *Streptococcus pyogenes*, three specific Lpps designated HtsA, FtsB and MtsA are important in iron transportation, and the transcription levels of those genes were increased in iron-limited environments (Lei et al. 2003; Hanks et al. 2005; Smoot et al. 2001; Janulczyk, Pallon, and Bjorck 1999). In another bacteria, *Streptococcus pneumoniae*, a trio of Lpps denoted pneumococcal iron acquisition, pneumococcal iron uptake, and pneumococcal iron transporter, function as substrate-binding proteins. These are critically involved in iron acquisition, and bacteria displayed attenuated growth or diminished virulence in murine infection models when utilising mutant strains of these specific Lpps in comparison to their parental strains (Brown, Gilliland, and Holden 2001; Brown et al. 2002). A pneumococcal mutant strain defective in all three Lpps exhibited growth defects *in vitro* in a variety of iron-restricted conditions, severely decreased the $^{55}FeCl_3$ accumulation, and reduced the sensitivity towards streptonigrin (Brown et al. 2002). In addition, a *Streptococcus pneumoniae* Δ*lgt* mutant exhibited reduced bacterial proliferation *in vitro* in cation-depleted conditions, impaired the acquisition of several cations, including iron, and resulted in growth defects in human blood and bronchoalveolar lavage fluid obtained from mice (Chimalapati et al. 2012). Furthermore, in *Streptococcus agalactiae*, the *in vitro* bacterial growth was impaired in Lpp mutants of Δ*lgt*, Δ*lsp* or Δ*lgt*/Δ*lsp* compared to their parental strain in nutrient-poor medium, indicating the importance of Lpp in metabolic fitness of this bacterium under restrictive conditions (Henneke et al. 2008).

Deletions of *lgt* and *lsp* genes are known to promote the release of unprocessed and defective Lpps in *S. aureus* (Stoll et al. 2005; Nguyen and Gotz 2016). It was previously revealed that substantial loss of Lpp was detected from the cytoplasmic membrane upon deletion of the *lsp* gene in Gram-positive *Streptomyces coelicolor* (Thompson et al. 2010). This Δ*lsp* mutant was also associated with inhibited bacterial growth as well as with formation of uncharacteristic small-sized colonies in contrast to its parental strain (Thompson et al. 2010).

In contrast to iron-restricted conditions, deletion of *lgt* tends to have minor effects on the bacterial growth in enriched- or complete media in various

Gram-positive bacteria, strongly suggesting that bacterial viability is mostly hampered only under stressful conditions and nutrient limitation. This has been reported for example in *S. aureus* (**Paper II**) (Mohammad et al. 2020; Stoll et al. 2005), as well as in other Gram-positive bacteria, such as *Streptococcus pneumoniae* (Petit et al. 2001; Chimalapati et al. 2012), *Streptococcus agalactiae* (Henneke et al. 2008; Bray, Sutcliffe, and Harrington 2009), *Streptococcus sanguinis* (Das et al. 2009), *Streptococcus suis* (Wichgers Schreur et al. 2011), *Streptococcus mutans* (Arimoto and Igarashi 2008), *Streptococcus equi* (Hamilton et al. 2006), *Bacillus subtilis* (Leskela et al. 1999), and *Listeria monocytogenes* (Baumgartner et al. 2007). In *Streptococcus gordonii*, a Δ*lgt* mutant exhibited a similar growth pattern as its parental strain, and did neither alter the size nor the cell morphology, while assessed by electron microscopy (Kim et al. 2018).

Earlier reports have indicated that the *lsp* gene is of less importance in numerous Gram-positive bacteria with regard to ability to grow *in vitro* as *lsp*-deficient mutants displayed similar growth rates to those of their parental strains (de Greeff et al. 2003; Sander et al. 2004; Venema et al. 2003; Reglier-Poupet et al. 2003; Tjalsma et al. 1999). Yet, in Gram-negative bacteria, such as *Salmonella typhimurium* and *Escherichia coli*, *lgt*- or *lsp*-deficient mutants result in fatal outcomes for the bacteria (Gan et al. 1993; Yamagata et al. 1983), underlining the critical role of Lpp maturation in the survival of Gram-negative bacteria (Hutchings et al. 2009).

5.4 *S. aureus* Lpp – *in vitro* effects

The use of *S. aureus* Δ*lgt* mutants as well as purified Lpp compounds and synthetic lipopeptides have demonstrated Lpps important role in triggering the release of cytokines and chemokines as a response of host immune defence mechanisms.

Synthetic lipopeptides are well-known to be potent stimulators of human monocytes and macrophages with subsequent secretion of various cytokines (Kreutz et al. 1997; Hoffmann et al. 1988). *S. aureus* Δ*lgt* mutants are associated with impaired production of pro-inflammatory cytokines and chemokines, including IL-6, IL-8, and MCP-1, in various human cells, such as the human monocytic cell line (MonoMac6), the human pulmonary epithelial

cell line (A549), and in human umbilical vein endothelial cells, in contrast to its parental strain (Stoll et al. 2005). Furthermore, both TNFα and IL-10 levels were diminished over time in MonoMac6 cells (Stoll et al. 2005). In another human monocytic cell line, referred to as THP-1 cells, cell stimulation with heat-killed *S. aureus* Δ*lgt* mutant was associated with lower production of TNF, IL-1β and IL-8, in contrast to those cells that were stimulated with the heat-killed *S. aureus* parental strain (Kang et al. 2011). Also, *S. aureus* Δ*lgt* mutants were associated with impaired TLR2-MyD88-mediated cytokine production (IL-1, IL-6, IL-10 and TNF) in mouse peritoneal macrophages, whereas the *S. aureus* parental strain induced early and strong cytokine release (Schmaler et al. 2009). Purified *S. aureus* SitC is well-known to induce TNF and IL-6 expression in murine peritoneal macrophages in a TLR2-MyD88 dependent manner (Kurokawa et al. 2009). The release of such pro-inflammatory cytokines was also demonstrated in human monocytes and mouse keratinocytes, and in TLR2 expressing HEK cells (Muller et al. 2010).

Our results show that purified *S. aureus* Lpp, Lpl1, upon stimulation of peritoneal macrophages induced a quick and dose-dependent release of the neutrophil-chemoattractant, MIP-2 and KC as well as monocyte-chemoattractant MCP-1 (**Paper I**) (Mohammad et al. 2019). These strong and rapid effects were observed already four hours after stimulation, and were dependent on the lipid- and not the protein-moiety. In fact, purified Lpl1(+sp), expressing Lpp, induced similar levels as the positive control, while Lpl1(-sp), lacking the lipid-moiety, was only capable to exhibit similar stimulation levels as the negative control for the assessed chemokine (**Paper I**) (Mohammad et al. 2019). These findings were only observed in the peritoneal macrophage supernatants collected from the C57BL/6 wild-type and not in the TLR2 deficient mice, and were thus mediated through the PRR, TLR2 (**Paper I**) (Mohammad et al. 2019). The importance of *S. aureus* Lpp and its lipidation was further verified by the fact that the triacylated lipid-moiety presented in Pam3CSK4 synthetic lipopeptide induced similar MIP-2, KC and MCP-1 levels as the purified Lpl1(+sp) compound (**Paper I**) (Mohammad et al. 2019). In addition to the neutrophil- and monocyte chemoattractant chemokines, the pro-inflammatory cytokine, TNFα, was also induced in a TLR2-dependent manner upon Lpl1(+sp) and Pam3CSK4 stimulation in both mouse peritoneal macrophages and in splenocyte cultures (**Paper I**) (Mohammad et al. 2019). Overall, our findings indicate that *S. aureus* Lpps are potent immune

stimulators that induce rapid release of important chemokines and cytokines from immune cells exclusively via TLR2.

On the other hand, our data revealed that SA113 parental strain and Δ*lgt* mutant strain proliferated similarly in mouse whole blood during a two hour incubation (**Paper I**) (Mohammad et al. 2019), which are not in corroboration to previous findings in human whole blood (Bubeck Wardenburg, Williams, and Missiakas 2006). However, parameters such as the assessed time points, different *S. aureus* strains (SA113 vs. Newman), and the different sources of whole blood (mouse vs. human) might explain these discrepancies. Nevertheless, in the model of systemic infection, *S. aureus* Lpp was attributable in inducing a more systemic inflammatory status, whereby the Newman parental strain was associated with higher levels of IL-6 and KC, but not MCP-1 (**Paper II**) (Mohammad et al. 2020). No differences between the parental strain and the *lgt* deficient mutant strain were observed in TLR2 knockout mice, suggesting that the cytokine response was dependent on TLR2 (**Paper II**) (Mohammad et al. 2020). Although *S. aureus* Newman parental strain had higher bacterial proliferation in the presence of activated murine macrophages compared with Newman Δ*lgt* mutant strain, the phagocytic capacity of macrophages was not affected by the Lpp expression (Bubeck Wardenburg, Williams, and Missiakas 2006). These results are in corroboration with our data whereby purified *S. aureus* Lpl1 did not influence the phagocytic capacity of macrophages (**Paper I**) (Mohammad et al. 2019).

Gardiner *et al*. studied the importance of *lnsAB* on the response of immune stimulation and demonstrated using HEK-TLR2 cells that the expression of IL-8 increased approximately 10 times upon deletion of either of the two genes, *lnsA* or *lnsB* (Gardiner et al. 2020). This suggests that an intact *lnsAB* system is advantageous for *S. aureus* in evading TLR2 immune recognition. Furthermore, various staphylococcal species possess varying lengths of the *N*-acylation at the N termini of the cysteine residue within the Lpp lipid-moiety: the commensal pathogens *S. aureus* and *S. epidermidis* carry a long-chain *N*-acylated fatty acid, while the non-commensal, non-pathogenic *S. carnosus* (Rosenstein and Gotz 2013; Biswas et al. 2009) carries a short-chain *N*-acylated fatty acid, which corresponds to a heptadecanoyl fatty acid and a acetyl fatty acid, respectively (Nguyen et al. 2017). Nguyen *et al*. revealed that *S. carnosus* was capable to induce ten-fold higher TLR2-mediated cytokine responses compared to the observed levels that were induced by *S. aureus* and

S. epidermidis (Nguyen et al. 2017). Furthermore, both TNFα and IL-8 secretion were strongly upregulated by *S. carnosus* in MonoMac6 and HEK-TLR2 cells in comparison with several *S. aureus* strains, including SA113, HG003, and the MRSA strain, USA300 (Nguyen et al. 2017). This concept was further proved as purified SitC Lpp extracted from *S. carnosus* yielded higher induction of TLR2-mediated IL-8 chemokine in contrast to the SitC Lpp that was extracted from *S. aureus* (Nguyen et al. 2017). This was also confirmed in human monocyte-derived dendritic cells whereby the levels of various pro-inflammatory cytokines were elevated in a similar manner (Nguyen et al. 2017). Overall, these findings are in line with previous reports that showed that different modifications of the lipid-moiety display diverse TLR2 activations (Armbruster, Komazin, and Meredith 2019; Gardiner et al. 2020). Interestingly, the expression of other *S. aureus* components, more precisely CP can mask *S. aureus* Lpps and attenuate the Lpp recognition and TLR2 activity (Hilmi et al. 2014).

It was recently demonstrated in HeLa cells that *lpl* from the νSaα island alters the host cell cycle, delays its transition, which consequently leads to increased cell invasion (Nguyen et al. 2016). This effect was shown to be mediated by the secretion of cyclomodulin (Nguyen et al. 2016), which functions as bacterial toxin that is known to disturb the regular course of the host cell cycle (Taieb, Nougayrede, and Oswald 2011). Cyclomodulin seems to be utilised by *S. aureus* as a part of its invasive nature as well as to induce bacterial proliferation within the host cells (Alekseeva et al. 2013). Several elements that play a role in the involvement of *lpl* and its subsequent invasiveness were recently addressed by Nguyen and colleagues (Nguyen et al. 2018). In fact, it was revealed that not only the bacterial growth phases of *S. aureus* play an important role but also that the host side contributes to such effects since TLR2 expressing cells promoted higher invasive frequencies (Nguyen et al. 2018).

Nguyen *et al* showed that deletion of the entire *lpl* operon in *S. aureus* USA300 strain resulted in attenuated induction of pro-inflammatory cytokines in human monocytes, macrophages and keratinocytes, in contrast to the parental strain expressing the *lpl* gene cluster (Nguyen et al. 2015). To verify the observed phenomenon, the *lpl* cluster was cloned into another *S. aureus* strain, HG003, which naturally lacks *lpl* genes, and this resulted in increased production of the pro-inflammatory cytokines (Nguyen et al. 2015). The purified lipidated Lpp, Lpl1, was further shown to evoke a TLR2-dependent response (Nguyen et al.

2015). A recent study revealed that the cluster of *lpl* proteins in *S. aureus* were upregulated in a MRSA strain during sub-inhibitory exposure to β-lactam antibiotics (Shang et al. 2019). This suggests that *S. aureus* Lpp, and more specifically *lpl*, has characteristics of virulent nature that *S. aureus* can utilise in order to enhance MRSA pathogenicity.

5.5 Lpp – *in vivo* effects: the role in virulence and pathogenicity

Besides its vital role in maintaining and upregulating the fitness of the bacteria (Shahmirzadi, Nguyen, and Gotz 2016), bacterial Lpp possesses a variety of key functions, some of which serve critical roles during infectious and inflammatory conditions. In most, but not all cases, maturation of Lpp has been strongly associated with enhanced pathogenic invasion, bacterial survival, and immune activation (Nguyen and Gotz 2016; Shahmirzadi, Nguyen, and Gotz 2016).

We reported that *S. aureus* Lpps play various roles in different murine models (**Paper I-III**). In a local knee arthritis model, *S. aureus* Lpps mediated severe arthritogenic effects in NMRI and C57BL/6 wild-type mice, but not in TLR2 deficient mice after intra-articular knee joint challenge with the purified Lpp, Lpl1(+sp) (**Paper I**) (Mohammad et al. 2019). The arthritogenic properties were mediated through the lipid-moiety, since Lpl1(-sp), comprising only of the protein-moiety, lacked the ability to cause any knee joint swelling. In addition to the observed long-lasting macroscopic arthritic effect in the Lpl1(+sp) group, which was detected already within 24 hours post-injection, Lpl1 contributed to local knee synovitis in a dose-dependent fashion. The histological sections revealed that Lpl1 induced synovitis even when administered at the nanogram level, indicating that *S. aureus* Lpps are highly potent. Intriguingly, when challenging the mice with an Lpl1 dose at the microgram level, all joints developed bone erosions within 10 days post-injection (**Paper I**) (Mohammad et al. 2019).

Prior to this study, we reported that murine knee joints, challenged with antibiotic-killed *S. aureus*, displayed severe bone erosion and long-lasting arthritis (Ali, Zhu, et al. 2015). This aspect is indeed very clinically relevant since patients suffering of septic arthritis are likely to develop irreversible

permanent joint destruction, even after successful bacterial eradication through the standard treatment procedure (Goldenberg 1998). Since the *S. aureus* cell wall components were responsible for causing this inflammatory effect in the joints, partially via TLR2 (Ali, Zhu, et al. 2015), this prompted us to study the significance of *S. aureus* Lpps as we hypothesised that Lpps might be one of the inducers. Indeed, we were able to verify this hypothesis, and also elucidated the cellular mechanism by showing that monocytes/macrophages were the responsible cell type mediating the Lpl1-induced effect in the local knee joints through TLR2-dependent mechanisms (**Paper I**) (Mohammad et al. 2019). This further strengthens the concept that the disease severity of septic arthritis is at least partially mediated by an exaggerated immune response, which is triggered by specific bacterial components, such as Lpp (**Paper I**) (Mohammad et al. 2019), which is in corroboration with previous reports (Ali, Zhu, et al. 2015; Deng et al. 1999).

In a local knee septic arthritis model triggered by intra-articular injection of living bacteria, SA113 Δ*lgt* mutant strain induced more knee swelling compared to its parental strain, which coincided with higher bacterial burden in the local knee and increased IL-6 expression (**Paper I**) (Mohammad et al. 2019). In contrast, in a murine sepsis model, lower bacterial loads were found in knee joints of mice who received intravenous injection of the same strain (Schmaler et al. 2009), which once again suggests that Lpps behave differently in different murine models, depending on the route of infection, the organ of interest, and the assessed time points. In fact, in our haematogenous septic arthritis model, increased bacterial persistence was observed in both C57BL/6 wild-type and TLR2 deficient murine kidneys when inoculated intravenously with the Newman parental strain (**Paper II**) (Mohammad et al. 2020). This is in agreement with earlier reports showing that different organs, including the kidneys, exhibited higher bacterial loads when infected with SA113 parental strain in comparison to the Δ*lgt* mutant strain, independent of TLR2 and MyD88 signalling (Schmaler et al. 2009). In addition, USA300 MRSA parental strain led to higher bacterial burden in kidneys of Balb/c mice compared to its Δ*lpl* mutant strain, lacking the *lpl* gene cluster (Nguyen et al. 2015).

Lpp was also shown to enhance the invasiveness of *S. aureus* in skin infection by displaying higher bacterial counts when mice were epicutaneously infected with the USA300 parental strain (Nguyen et al. 2015). A similar phenomenon was observed when we subcutaneously infected the mice with the SA113 or

the Newman strain (**Paper III**). This shows that different *S. aureus* Lpp-expressing strains give rise to similar virulent characteristics in two different skin infection models. In Gram-positive *Bacillus anthracis*, *lgt*-deficiency contributed to diminished virulence in mouse skin tissue (Okugawa et al. 2012). In another Gram-positive bacteria, *Streptococcus pyogenes*, skin infection of mice with an *lsp*-deficient strain led to impaired virulence with less pronounced skin lesions in comparison to its parental strain (Weston, Brenot, and Caparon 2009). Purified *S. aureus* Lpps were shown to promote murine skin inflammation through activation of dendritic cells in an intradermal injection model (Saito and Quadery 2018). Likewise, induced skin inflammation was also observed in our subcutaneous skin injection model (**Paper III**).

The cellular mechanism behind the lower bacterial load in the murine knee joints intra-articularly inoculated with Lpp-expressing *S. aureus* was elucidated using co-injection of live bacteria with purified *S. aureus* Lpp or synthetic lipopeptides. This resulted in bacterial eradication in the knee joints – a phenomenon that was mediated through TLR2-dependent mechanisms with neutrophils acting as the main phagocytic cell engulfing the bacteria (**Paper I**) (Mohammad et al. 2019). In fact, enhanced levels of the neutrophil attracting chemokines, KC and MIP-2, were observed in the supernatants of knee homogenates (**Paper I**) (Mohammad et al. 2019). Our data indicate that not only purified *S. aureus* Lpp but also Lpps immersed in live bacteria are able to activate a powerful innate immune response. The elevated levels of the same chemokines were also observed in the skin homogenates upon subcutaneous injection with purified *S. aureus* Lpp (**Paper III)**, strongly suggesting that Lpps play a potent role in triggering local inflammatory responses in different organs. In a murine sepsis model, it was recently demonstrated that mice pre-treated with the synthetic lipopeptide, Pam3CSK4, had decreased bacterial burden and increased survival following infection with a *S. aureus* MRSA strain (Huang et al. 2017).

In the haematogenous septic arthritis model, expression of Lpp in *S. aureus* increases mortality, weight loss, and cytokine production and decreases bacterial clearance independent of TLR2, indicating the important role of Lpp expression in bacterial fitness and virulence (**Paper II**) (Mohammad et al. 2020). TLR2, as a Lpp receptor, plays a role in the host defence against infection, as TLR2 deficient mice infected with the Newman parental strain

displayed enhanced arthritis symptoms as well as increased weight loss, mortality and bacterial burden in kidneys compared to the wild-type controls (**Paper II**) (Mohammad et al. 2020). This result was not so surprising since several reports previously showed that TLR2 deficient mice are significantly more susceptible to *S. aureus*-induced infections and display increased bacterial loads in different organs in comparison to their wild-type counterparts (Schmaler et al. 2009; Stenzel et al. 2008; Miller et al. 2006; Sun et al. 2006; Takeuchi, Hoshino, and Akira 2000).

In the local knee arthritis model, we demonstrated that the destructive arthritis caused by Lpp is TLR2-dependent (**Paper I**) (Mohammad et al. 2019); possibly due to an excessive inflammatory reaction. However, in the haematogenous septic arthritis model, we showed that the destructive arthritis caused by Lpp-expressing *S. aureus* was TLR2-independent. Multi-functions of Lpp i.e. maintaining the metabolic nutrition and fitness, bacterial survival and pathogenicity during host-interactions, or its ability to evade immune recognition or to trigger various immune responses upon invasion are all of significant importance during infection with live *S. aureus* expressing Lpp.

Our findings from the murine haematogenous septic arthritis model and from the local knee arthritis model are illustrated in Figure 5.

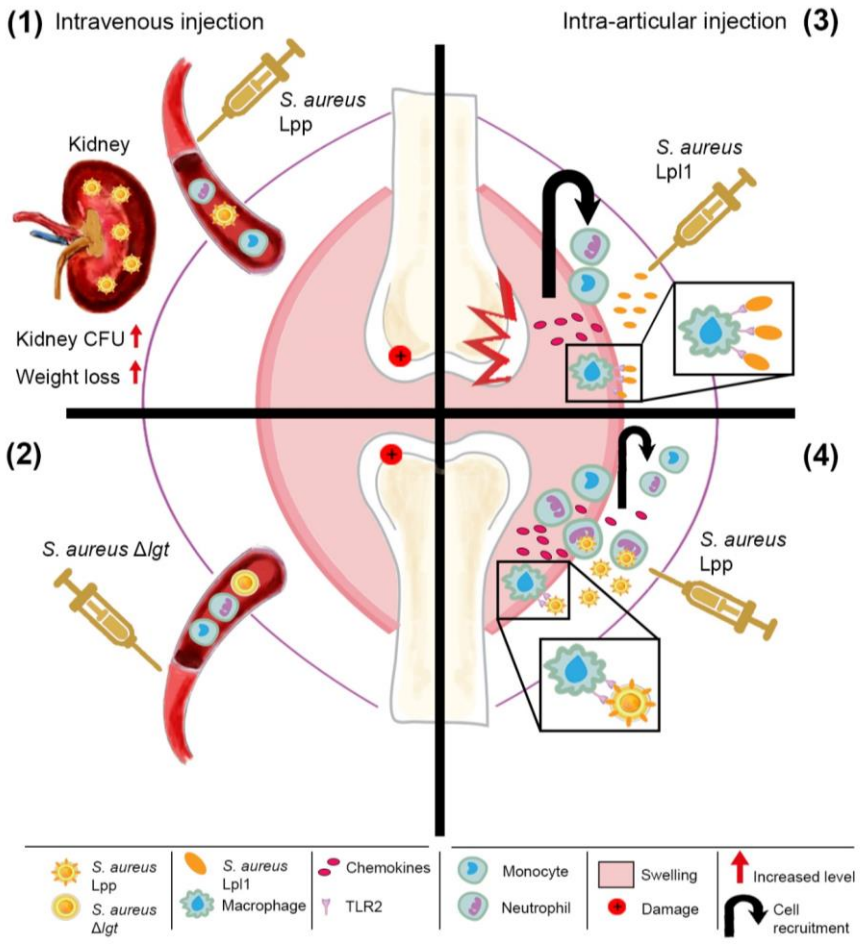


Figure 5. Schematic illustration of S. aureus lipoprotein (Lpp) effects in haematogenous and local S. aureus arthritis models.
*Left panel: S. aureus parental strain, expressing Lpp (S. aureus Lpp), significantly aggravated systemic infection with increased mortality, weight loss, and bacterial burden in the kidneys (**1**) compared to its lgt mutant strain, lacking Lpp (S. aureus Δlgt) (**2**). However, both S. aureus strains had similar outcomes with regard to bone erosion. Right panel: Lpp had dual effects in the local knee model. Intra-articular injection of purified Lpp (S. aureus Lpl1) induced rapid TLR2-dependent infiltration of phagocytes. Moreover, Lpl1 caused severe joint inflammation and bone erosions dependent on monocytes/macrophages through TLR2 (**3**). On the other hand, live S. aureus Lpp acted as adjuvants, triggering recognition by TLR2 and subsequent neutrophil recruitment, leading to more efficient bacterial killing and diminished bone destruction (**4**). CFU = colony forming units.*

Lpps have emerged as an important factor during the pathogenesis of *S. aureus* systemic infections (**Paper II**) (Schmaler et al. 2009; Mohammad et al. 2020). This was further strengthened in other Gram-positive bacteria. Petit *et al*. showed that Lpp from *Streptococcus pneumoniae* was imperative for the bacteria to initiate and maintain infection in an intranasal model (Petit et al. 2001). In addition, deletion of the *lgt* gene in *Streptococcus pneumoniae* was associated with significantly attenuated virulence in different animal models such as pneumonia, sepsis, and significantly diminished nasopharyngeal colonization of the mice (Chimalapati et al. 2012). In another Gram-positive bacteria, *Streptococcus gordonii*, mice receiving the strain lacking Lpp had increased survival compared to the mice who received the parental strain expressing Lpp, further illustrating the pathogenic role of Lpp (Kim et al. 2018).

In Gram-positive *Streptococcus equi*, lack of Lpp markedly ameliorated disease severity in a mouse but not horse model of strangles, an upper tract infectious disease that is mostly prevalent in horses (Hamilton et al. 2006). Furthermore, in a sepsis model of *Listeria monocytogenes*, Lpps were found to be critical virulence determinants. Mice infected with the strain expressing Lpp succumbed to disease within few days while none of the mice in the group infected with a strain lacking Lpp died (Machata et al. 2008). In *Mycobacterium tuberculosis*, Lpp was also shown to be a virulent factor in a tuberculosis model. Using a mutant strain, lacking the *lsp* enzyme, significantly attenuated disease severity, and the observed results were independent of the genetic background of the mice (Sander et al. 2004).

However, there are very few studies showing contrasting results. In Gram-positive *Streptococcus agalactiae*, Lpp mediated protection via TLR2 with increased survival compared to its *lgt*-deficient strain in a murine sepsis model (Henneke et al. 2008). In addition, another study indicated that *S. aureus* Δ*lgt* mutant was hypervirulent in contrast to its parental strain (Bubeck Wardenburg, Williams, and Missiakas 2006). Surprisingly, in the same article, the authors demonstrated that the *lsp*-deficient strain exhibited attenuated virulence (Bubeck Wardenburg, Williams, and Missiakas 2006), which is more in line with many other studies. One could speculate that these contradictory findings might be due to variations in animal species and age, bacterial dose or bacterial strain, duration of the course of infection, or that the generation of the *lgt* deletion mutant might have been processed differently in the reported

studies, which might convey these divergent outcomes on lipidation and maturation of staphylococcal Lpp in murine staphylococcal sepsis. Nevertheless, our conclusion from the majority of the previous studies is that bacterial Lpps are pathogenic in systemic and skin infections. Gram-positive bacterial Lpps and their differential roles in different *in vivo* models are summarised in Table 2.

Table 2. Gram-positive bacterial lipoproteins and their distinct role in different *in vivo* animal models.

Site/organ - administration	Species/compound	Outcome	References
	Murine models		
Knee – intra-articular	Purified *S. aureus* Lpp	- Induced bone destruction	**Paper I** (Mohammad et al., 2019)
Knee – intra-articular	*S. aureus Δlgt*	- Knee swelling ↑ - CFU ↑	**Paper I** (Mohammad et al., 2019)
Knee – intra-articular (co-injection)	Purified *S. aureus* Lpp + *S. aureus*	- Bone destruction ↓ - Bacterial load ↓	**Paper I** (Mohammad et al., 2019)
Septic arthritis - intravenous	*S. aureus Δlgt*	- Virulence ↓ - Arthritis severity- no effect	**Paper II** (Mohammad et al., 2020)
Sepsis – intravenous	*S. aureus Δlgt*	- Virulence ↓	(Schmaler et al., 2009)
Sepsis – intravenous	*S. aureus Δlgt*	- Virulence ↑	(Bubeck Wardenburg et al., 2006)
Sepsis – intravenous	*S. aureus Δlsp*	- Virulence ↓	(Bubeck Wardenburg et al., 2006)
Skin – subcutaneous	*S. aureus Δlgt*	- Virulence ↓	**Paper III** (Mohammad et al., 2020)
Skin – epicutaneous	*S. aureus Δlpl*	- Virulence ↓	(Nguyen et al., 2015)
Skin – intradermal	Purified *S. aureus* Lpp	- Virulence ↑	(Saito and Quadery, 2018)
Skin – subcutaneous	*Bacillus anthracis Δlgt*	- Virulence ↓	(Okugawa et al., 2012)
Skin – subcutaneous	*Streptococcus pyogenes Δlsp*	- Virulence ↓	(Weston et al., 2009)
Sepsis – subcutaneous	*Streptococcus agalactiae Δlgt*	- Virulence ↑	(Henneke et al., 2008)
Pneumonia – intranasal	*Streptococcus pneumoniae Δlgt*	- Virulence ↓	(Petit et al., 2001)
Sepsis – intraperitoneal	*Streptococcus pneumoniae Δlgt*	- Virulence ↓	(Chimalapati et al., 2012)
Pneumonia – intranasal	*Streptococcus pneumoniae Δlgt*	- Virulence ↓	
Sepsis – intraperitoneal	*Streptococcus gordonii Δlgt*	- Virulence ↓	(Kim et al., 2018)
Respiratory tract infection - intranasal	*Streptococcus equi Δlgt*	- Virulence ↓	(Hamilton et al., 2006)
Sepsis – intravenous	*Listeria monocyto-genes Δlgt*	- Virulence ↓	(Machata et al., 2008)
Tuberculosis – intranasal	*Mycobacterium tuberculosis Δlsp*	- Virulence ↓	(Sander et al., 2004)
	Other models		
Pony, intranasal	*Streptococcus equi Δlgt*	- No difference	(Hamilton et al., 2006)

Lpp = lipoproteins, Δ*lgt* = deletion mutant of preprolipoprotein diacylglyceryl transferase, Δ*lsp* = deletion mutant of prolipoprotein signal peptidase.

6. Conclusion

S. aureus Lpps play a differential role depending on the affected organ and route of injection, as described below.

In **Paper I**, Lpps gave rise to a dual function in local *S. aureus* arthritis models. On the one hand, purified Lpp, but not TSST-1 or PGN, induced chronic macroscopic arthritis. Intra-articular injection with Lpps induced rapid TLR2-dependent infiltration of monocytes/macrophages and neutrophils. Furthermore, *S. aureus* Lpps caused severe joint inflammation and bone erosions, which were mediated by monocytes/macrophages through TLR2.
On the other hand, Lpp expression in *S. aureus* led to reduced bacterial burden in the arthritic knee joints. The observed phenomenon was due to Lpp acting as adjuvant and triggering recognition by TLR2 followed by subsequent neutrophil recruitment, leading to more efficient bacterial killing and diminished bone destruction.

In **Paper II**, *S. aureus* Lpps were found to be prominent virulence factors independent of host TLR2 expression. Mice that were intravenously inoculated with the *S. aureus* Lpp-expressing parental strain succumbed more to the disease, had increased weight loss, and exhibited impaired bacterial clearance in their kidneys, in comparison to the *S. aureus* Δ*lgt* mutant strain, lacking Lpp expression. Notably, the worst outcome was observed in mice lacking TLR2 and inoculated with the *S. aureus* parental strain, strongly indicating the protective role of TLR2 in haematogenous spread of *S. aureus*-induced septic arthritis. However, in contrast to the local septic arthritis model, *S. aureus* Lpp exhibited a limited role on bone erosion.

In **Paper III**, *S. aureus* Lpps were associated with severe inflammatory response in the skin model. The observed skin lesions and inflammation were mediated through TLR2-dependent mechanisms. Lpp contributed to the influx of innate immune cells in a similar manner as observed in **Paper I** with monocytes/macrophages as well as neutrophils recruited to the local tissue. In addition, subcutaneous injection of *S. aureus* parental strain was associated with elevated bacterial burden in the skin biopsies. Importantly, Lpp maturation contributed to staphylococcal immune evasion. The main findings of our results are summarised in **Paper III.**

7. Future perspectives

In **Paper I**, we investigated the role of *S. aureus* Lpp on the induction of bone erosion and clarified the cellular and molecular mechanism of how *S. aureus* Lpps induce joint inflammation and bone erosion in murine septic arthritis. Our next objective is to study Lpps impact on the bone mineral density in septic arthritis as the bone mineral density is known to be quickly reduced in mice with septic arthritis (Verdrengh et al. 2006). Bone mass measurements provide important information with regard to the patient's bone health and thus serve as an important clinical tool for diagnosing osteoporosis and determining the risk of bone fractures (Richards et al. 2008). Due to *S. aureus* Lpps' potent role in causing severe joint inflammation and focal bone damage in mice (**Paper I**), we plan to assess how this affects the bone loss over time as well as to elucidate whether different immune cell depletions influence the bone mineral density in Lpp-induced arthritis.

Furthermore, as mentioned above, there are two main different structures of lipid-moieties from bacterial Lpps; diacylated and triacylated Lpps. Our future aim is to go deeper and compare different lipid-moieties of the staphylococcal species, *S. aureus* and *S. carnosus*, as well as the synthetic lipopeptides, Pam3CSK4 and Pam2CSK4, and investigate their role in the induction of bone erosion. To our knowledge, there are currently no studies available regarding the role of di- and tri-acylated Lpps on the induction of bone damage. We hypothesise that the diacylated Lpp structure is more potent inducer of bone erosion. As outlined before, the degree of acylation of the lipid-moiety impacts the immune response. Importantly, Nguyen *et al*. recently showed that lipid-moieties of Lpps from different bacterial species significantly differ regarding their immune stimulatory activity (Nguyen et al. 2017). In addition, earlier studies conducted on skin resident cells demonstrated that di- but not triacylated Lpps suppressed the immune tolerance, a phenomenon that was mediated through IL-6 release, and the subsequent induction and accumulation of myeloid-derived suppressor cells (Skabytska et al. 2014).

Finally, it has recently been proposed that combination of different staphylococcal MAMPs might exert an additive or possibly even a synergistic effect in immune stimulation (Nguyen and Gotz 2016). *S. aureus* Lpps as well

as PGN, are known MAMPs of *S. aureus* (Nguyen and Gotz 2016). It is known that most staphylococcal infections are successfully promoted by coordinated action of different virulence factors rather than a single virulence factor (Fournier and Philpott 2005). In fact, co-stimulation of dendritic cells with PGN and synthetic lipopeptide, enhanced immune stimulatory effects compared to PGN or lipopeptide stimulation alone (Schaffler et al. 2014). Therefore, we plan to determine whether Lpps and PGN act synergistically in staphylococcal skin infections.

As we have seen through the above studies, Lpp gave rise to different outcomes in different organs. What does this depend on? We speculate that the different clinical outcomes might be explained by the anatomic differences, composition difference of immune cells and distribution of blood vessels in the different organs. However, more detailed studies are warranted in the future to answer this question.

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REFERENCES

Abdelnour, A., S. Arvidson, T. Bremell, C. Ryden, and A. Tarkowski. 1993. 'The accessory gene regulator (agr) controls Staphylococcus aureus virulence in a murine arthritis model', *Infect Immun*, 61: 3879-85.

Abdelnour, A., T. Bremell, R. Holmdahl, and A. Tarkowski. 1994. 'Role of T lymphocytes in experimental Staphylococcus aureus arthritis', *Scand J Immunol*, 39: 403-8.

Abdelnour, A., T. Bremell, and A. Tarkowski. 1994. 'Toxic shock syndrome toxin 1 contributes to the arthritogenicity of Staphylococcus aureus', *J Infect Dis*, 170: 94-9.

Adler, A., V. Temper, C. S. Block, N. Abramson, and A. E. Moses. 2006. 'Panton-Valentine leukocidin-producing Staphylococcus aureus', *Emerg Infect Dis*, 12: 1789-90.

Akira, S., S. Uematsu, and O. Takeuchi. 2006. 'Pathogen recognition and innate immunity', *Cell*, 124: 783-801.

Alekseeva, L., L. Rault, S. Almeida, P. Legembre, V. Edmond, V. Azevedo, A. Miyoshi, S. Even, F. Taieb, Y. Arlot-Bonnemains, Y. Le Loir, and N. Berkova. 2013. 'Staphylococcus aureus-induced G2/M phase transition delay in host epithelial cells increases bacterial infective efficiency', *PLoS One*, 8: e63279.

Ali, A., M. Na, M. N. Svensson, M. Magnusson, A. Welin, J. C. Schwarze, M. Mohammad, E. Josefsson, R. Pullerits, and T. Jin. 2015. 'IL-1 Receptor Antagonist Treatment Aggravates Staphylococcal Septic Arthritis and Sepsis in Mice', *PLoS One*, 10: e0131645.

Ali, A., A. Welin, J. C. Schwarze, M. N. Svensson, M. Na, A. Jarneborn, M. Magnusson, M. Mohammad, J. Kwiecinski, E. Josefsson, J. Bylund, R. Pullerits, and T. Jin. 2015. 'CTLA4 Immunoglobulin but Not Anti-Tumor Necrosis Factor Therapy Promotes Staphylococcal Septic Arthritis in Mice', *J Infect Dis*, 212: 1308-16.

Ali, A., X. Zhu, J. Kwiecinski, I. Gjertsson, C. Lindholm, Y. Iwakura, X. Wang, N. Lycke, E. Josefsson, R. Pullerits, and T. Jin. 2015. 'Antibiotic-killed Staphylococcus aureus induces destructive arthritis in mice', *Arthritis Rheumatol*, 67: 107-16.

Aliprantis, A. O., R. B. Yang, M. R. Mark, S. Suggett, B. Devaux, J. D. Radolf, G. R. Klimpel, P. Godowski, and A. Zychlinsky. 1999. 'Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2', *Science*, 285: 736-9.

Allard, M., H. Moisan, E. Brouillette, A. L. Gervais, M. Jacques, P. Lacasse, M. S. Diarra, and F. Malouin. 2006. 'Transcriptional modulation of some Staphylococcus aureus iron-regulated genes during growth in vitro and in a tissue cage model in vivo', *Microbes Infect*, 8: 1679-90.

Alonzo, F., 3rd, M. A. Benson, J. Chen, R. P. Novick, B. Shopsin, and V. J. Torres. 2012. 'Staphylococcus aureus leucocidin ED contributes to

systemic infection by targeting neutrophils and promoting bacterial growth in vivo', *Mol Microbiol*, 83: 423-35.

Argudin, M. A., M. C. Mendoza, and M. R. Rodicio. 2010. 'Food poisoning and Staphylococcus aureus enterotoxins', *Toxins (Basel)*, 2: 1751-73.

Arimoto, T., and T. Igarashi. 2008. 'Role of prolipoprotein diacylglyceryl transferase (Lgt) and lipoprotein-specific signal peptidase II (LspA) in localization and physiological function of lipoprotein MsmE in Streptococcus mutans', *Oral Microbiol Immunol*, 23: 515-9.

Armbruster, K. M., G. Komazin, and T. C. Meredith. 2019. 'Copper-Induced Expression of a Transmissible Lipoprotein Intramolecular Transacylase Alters Lipoprotein Acylation and the Toll-Like Receptor 2 Response to Listeria monocytogenes', *J Bacteriol*, 201.

———. 2020. 'Bacterial lyso-form lipoproteins are synthesized via an intramolecular acyl chain migration', *J Biol Chem*, 295: 10195-211.

Armbruster, K. M., and T. C. Meredith. 2017. 'Identification of the Lyso-Form N-Acyl Intramolecular Transferase in Low-GC Firmicutes', *J Bacteriol*, 199.

Asanuma, M., K. Kurokawa, R. Ichikawa, K. H. Ryu, J. H. Chae, N. Dohmae, B. L. Lee, and H. Nakayama. 2011. 'Structural evidence of alpha-aminoacylated lipoproteins of Staphylococcus aureus', *FEBS J*, 278: 716-28.

Askarian, F., T. Wagner, M. Johannessen, and V. Nizet. 2018. 'Staphylococcus aureus modulation of innate immune responses through Toll-like (TLR), (NOD)-like (NLR) and C-type lectin (CLR) receptors', *FEMS Microbiol Rev*, 42: 656-71.

Baba, T., T. Bae, O. Schneewind, F. Takeuchi, and K. Hiramatsu. 2008. 'Genome sequence of Staphylococcus aureus strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands', *J Bacteriol*, 190: 300-10.

Babu, M. M., M. L. Priya, A. T. Selvan, M. Madera, J. Gough, L. Aravind, and K. Sankaran. 2006. 'A database of bacterial lipoproteins (DOLOP) with functional assignments to predicted lipoproteins', *J Bacteriol*, 188: 2761-73.

Baranwal, G., M. Mohammad, A. Jarneborn, B. R. Reddy, A. Golla, S. Chakravarty, L. Biswas, F. Gotz, S. Shankarappa, T. Jin, and R. Biswas. 2017. 'Impact of cell wall peptidoglycan O-acetylation on the pathogenesis of Staphylococcus aureus in septic arthritis', *Int J Med Microbiol*, 307: 388-97.

Barton, G. M., and R. Medzhitov. 2003. 'Toll-like receptor signaling pathways', *Science*, 300: 1524-5.

Baumgartner, M., U. Karst, B. Gerstel, M. Loessner, J. Wehland, and L. Jansch. 2007. 'Inactivation of Lgt allows systematic characterization of lipoproteins from Listeria monocytogenes', *J Bacteriol*, 189: 313-24.

Bera, A., S. Herbert, A. Jakob, W. Vollmer, and F. Gotz. 2005. 'Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of Staphylococcus aureus', *Mol Microbiol*, 55: 778-87.

Bhakdi, S., and J. Tranum-Jensen. 1991. 'Alpha-toxin of Staphylococcus aureus', *Microbiol Rev*, 55: 733-51.

Biswas, L., R. Biswas, C. Nerz, K. Ohlsen, M. Schlag, T. Schafer, T. Lamkemeyer, A. K. Ziebandt, K. Hantke, R. Rosenstein, and F. Gotz. 2009. 'Role of the twin-arginine translocation pathway in Staphylococcus', *J Bacteriol*, 191: 5921-9.

Braun, V. 2001. 'Iron uptake mechanisms and their regulation in pathogenic bacteria', *Int J Med Microbiol*, 291: 67-79.

Braun, V., and K. Rehn. 1969. 'Chemical characterization, spatial distribution and function of a lipoprotein (murein-lipoprotein) of the E. coli cell wall. The specific effect of trypsin on the membrane structure', *Eur J Biochem*, 10: 426-38.

Brauweiler, A. M., E. Goleva, and D. Y. M. Leung. 2019. 'Staphylococcus aureus Lipoteichoic Acid Damages the Skin Barrier through an IL-1-Mediated Pathway', *J Invest Dermatol*, 139: 1753-61 e4.

Bray, B. A., I. C. Sutcliffe, and D. J. Harrington. 2009. 'Impact of lgt mutation on lipoprotein biosynthesis and in vitro phenotypes of Streptococcus agalactiae', *Microbiology*, 155: 1451-58.

Bremell, T., A. Abdelnour, and A. Tarkowski. 1992. 'Histopathological and serological progression of experimental Staphylococcus aureus arthritis', *Infect Immun*, 60: 2976-85.

Brightbill, H. D., D. H. Libraty, S. R. Krutzik, R. B. Yang, J. T. Belisle, J. R. Bleharski, M. Maitland, M. V. Norgard, S. E. Plevy, S. T. Smale, P. J. Brennan, B. R. Bloom, P. J. Godowski, and R. L. Modlin. 1999. 'Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors', *Science*, 285: 732-6.

Brown, J. S., S. M. Gilliland, and D. W. Holden. 2001. 'A Streptococcus pneumoniae pathogenicity island encoding an ABC transporter involved in iron uptake and virulence', *Mol Microbiol*, 40: 572-85.

Brown, J. S., S. M. Gilliland, J. Ruiz-Albert, and D. W. Holden. 2002. 'Characterization of pit, a Streptococcus pneumoniae iron uptake ABC transporter', *Infect Immun*, 70: 4389-98.

Brown, S., J. P. Santa Maria, Jr., and S. Walker. 2013. 'Wall teichoic acids of gram-positive bacteria', *Annu Rev Microbiol*, 67: 313-36.

Brulle, J. K., T. Grau, A. Tschumi, Y. Auchli, R. Burri, S. Polsfuss, P. M. Keller, P. Hunziker, and P. Sander. 2010. 'Cloning, expression and characterization of Mycobacterium tuberculosis lipoprotein LprF', *Biochem Biophys Res Commun*, 391: 679-84.

Bubeck Wardenburg, J., R. J. Patel, and O. Schneewind. 2007. 'Surface proteins and exotoxins are required for the pathogenesis of Staphylococcus aureus pneumonia', *Infect Immun*, 75: 1040-4.

Bubeck Wardenburg, J., W. A. Williams, and D. Missiakas. 2006. 'Host defenses against Staphylococcus aureus infection require recognition of bacterial lipoproteins', *Proc Natl Acad Sci U S A*, 103: 13831-6.

Buddelmeijer, N. 2015. 'The molecular mechanism of bacterial lipoprotein modification--how, when and why?', *FEMS Microbiol Rev*, 39: 246-61.

Cassat, J. E., and E. P. Skaar. 2013. 'Iron in infection and immunity', *Cell Host Microbe*, 13: 509-19.

Chambers, H. F., and F. R. Deleo. 2009. 'Waves of resistance: Staphylococcus aureus in the antibiotic era', *Nat Rev Microbiol*, 7: 629-41.

Cheng, A. G., A. C. DeDent, O. Schneewind, and D. Missiakas. 2011. 'A play in four acts: Staphylococcus aureus abscess formation', *Trends Microbiol*, 19: 225-32.

Cheng, A. G., H. K. Kim, M. L. Burts, T. Krausz, O. Schneewind, and D. M. Missiakas. 2009. 'Genetic requirements for Staphylococcus aureus abscess formation and persistence in host tissues', *FASEB J*, 23: 3393-404.

Cheng, A. G., M. McAdow, H. K. Kim, T. Bae, D. M. Missiakas, and O. Schneewind. 2010. 'Contribution of coagulases towards Staphylococcus aureus disease and protective immunity', *PLoS Pathog*, 6: e1001036.

Cheng, B. L., T. B. Nielsen, P. Pantapalangkoor, F. Zhao, J. C. Lee, C. P. Montgomery, B. Luna, B. Spellberg, and R. S. Daum. 2017. 'Evaluation of serotypes 5 and 8 capsular polysaccharides in protection against Staphylococcus aureus in murine models of infection', *Hum Vaccin Immunother*, 13: 1609-14.

Cheung, A. L., K. J. Eberhardt, E. Chung, M. R. Yeaman, P. M. Sullam, M. Ramos, and A. S. Bayer. 1994. 'Diminished virulence of a sar-/agr- mutant of Staphylococcus aureus in the rabbit model of endocarditis', *J Clin Invest*, 94: 1815-22.

Cheung, G. Y., H. S. Joo, S. S. Chatterjee, and M. Otto. 2014. 'Phenol-soluble modulins--critical determinants of staphylococcal virulence', *FEMS Microbiol Rev*, 38: 698-719.

Cheung, G. Y., R. Wang, B. A. Khan, D. E. Sturdevant, and M. Otto. 2011. 'Role of the accessory gene regulator agr in community-associated methicillin-resistant Staphylococcus aureus pathogenesis', *Infect Immun*, 79: 1927-35.

Chimalapati, S., J. M. Cohen, E. Camberlein, N. MacDonald, C. Durmort, T. Vernet, P. W. Hermans, T. Mitchell, and J. S. Brown. 2012. 'Effects of deletion of the Streptococcus pneumoniae lipoprotein diacylglyceryl transferase gene lgt on ABC transporter function and on growth in vivo', *PLoS One*, 7: e41393.

Claes, J., L. Liesenborghs, M. Peetermans, T. R. Veloso, D. Missiakas, O. Schneewind, S. Mancini, J. M. Entenza, M. F. Hoylaerts, R. Heying, P. Verhamme, and T. Vanassche. 2017. 'Clumping factor A, von

Willebrand factor-binding protein and von Willebrand factor anchor Staphylococcus aureus to the vessel wall', *J Thromb Haemost*, 15: 1009-19.

Claes, J., T. Vanassche, M. Peetermans, L. Liesenborghs, C. Vandenbriele, K. Vanhoorelbeke, D. Missiakas, O. Schneewind, M. F. Hoylaerts, R. Heying, and P. Verhamme. 2014. 'Adhesion of Staphylococcus aureus to the vessel wall under flow is mediated by von Willebrand factor-binding protein', *Blood*, 124: 1669-76.

Colavite, P. M., and A. Sartori. 2014. 'Septic arthritis: immunopathogenesis, experimental models and therapy', *J Venom Anim Toxins Incl Trop Dis*, 20: 19.

Cremieux, A. C., A. Saleh-Mghir, C. Danel, F. Couzon, O. Dumitrescu, T. Lilin, C. Perronne, J. Etienne, G. Lina, and F. Vandenesch. 2014. 'alpha-Hemolysin, not Panton-Valentine leukocidin, impacts rabbit mortality from severe sepsis with methicillin-resistant Staphylococcus aureus osteomyelitis', *J Infect Dis*, 209: 1773-80.

Dale, S. E., M. T. Sebulsky, and D. E. Heinrichs. 2004. 'Involvement of SirABC in iron-siderophore import in Staphylococcus aureus', *J Bacteriol*, 186: 8356-62.

Das, S., T. Kanamoto, X. Ge, P. Xu, T. Unoki, C. L. Munro, and T. Kitten. 2009. 'Contribution of lipoproteins and lipoprotein processing to endocarditis virulence in Streptococcus sanguinis', *J Bacteriol*, 191: 4166-79.

De Filippo, K., R. B. Henderson, M. Laschinger, and N. Hogg. 2008. 'Neutrophil chemokines KC and macrophage-inflammatory protein-2 are newly synthesized by tissue macrophages using distinct TLR signaling pathways', *J Immunol*, 180: 4308-15.

de Greeff, A., A. Hamilton, I. C. Sutcliffe, H. Buys, L. van Alphen, and H. E. Smith. 2003. 'Lipoprotein signal peptidase of Streptococcus suis serotype 2', *Microbiology*, 149: 1399-407.

DeLeo, F. R., and H. F. Chambers. 2009. 'Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era', *J Clin Invest*, 119: 2464-74.

DeLeo, F. R., M. Otto, B. N. Kreiswirth, and H. F. Chambers. 2010. 'Community-associated meticillin-resistant Staphylococcus aureus', *Lancet*, 375: 1557-68.

Deng, G. M., I. M. Nilsson, M. Verdrengh, L. V. Collins, and A. Tarkowski. 1999. 'Intra-articularly localized bacterial DNA containing CpG motifs induces arthritis', *Nat Med*, 5: 702-5.

Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. H. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Perdreau-Remington. 2006. 'Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant Staphylococcus aureus', *Lancet*, 367: 731-9.

Dryden, M. S. 2009. 'Skin and soft tissue infection: microbiology and epidemiology', *Int J Antimicrob Agents*, 34 Suppl 1: S2-7.

———. 2010. 'Complicated skin and soft tissue infection', *J Antimicrob Chemother*, 65 Suppl 3: iii35-44.

Dumitrescu, O., P. Choudhury, S. Boisset, C. Badiou, M. Bes, Y. Benito, C. Wolz, F. Vandenesch, J. Etienne, A. L. Cheung, M. G. Bowden, and G. Lina. 2011. 'Beta-lactams interfering with PBP1 induce Panton-Valentine leukocidin expression by triggering sarA and rot global regulators of Staphylococcus aureus', *Antimicrob Agents Chemother*, 55: 3261-71.

Dziewanowska, K., J. M. Patti, C. F. Deobald, K. W. Bayles, W. R. Trumble, and G. A. Bohach. 1999. 'Fibronectin binding protein and host cell tyrosine kinase are required for internalization of Staphylococcus aureus by epithelial cells', *Infect Immun*, 67: 4673-8.

Edwards, A. M., and R. C. Massey. 2011. 'How does Staphylococcus aureus escape the bloodstream?', *Trends Microbiol*, 19: 184-90.

Elasri, M. O., J. R. Thomas, R. A. Skinner, J. S. Blevins, K. E. Beenken, C. L. Nelson, and M. S. Smeltzer. 2002. 'Staphylococcus aureus collagen adhesin contributes to the pathogenesis of osteomyelitis', *Bone*, 30: 275-80.

Entenza, J. M., T. J. Foster, D. Ni Eidhin, P. Vaudaux, P. Francioli, and P. Moreillon. 2000. 'Contribution of clumping factor B to pathogenesis of experimental endocarditis due to Staphylococcus aureus', *Infect Immun*, 68: 5443-6.

Falugi, F., H. K. Kim, D. M. Missiakas, and O. Schneewind. 2013. 'Role of protein A in the evasion of host adaptive immune responses by Staphylococcus aureus', *mBio*, 4: e00575-13.

Fatima, F., Y. Fei, A. Ali, M. Mohammad, M. C. Erlandsson, M. I. Bokarewa, M. Nawaz, H. Valadi, M. Na, and T. Jin. 2017. 'Radiological features of experimental staphylococcal septic arthritis by micro computed tomography scan', *PLoS One*, 12: e0171222.

Fei, Y., W. Wang, J. Kwiecinski, E. Josefsson, R. Pullerits, I. M. Jonsson, M. Magnusson, and T. Jin. 2011. 'The combination of a tumor necrosis factor inhibitor and antibiotic alleviates staphylococcal arthritis and sepsis in mice', *J Infect Dis*, 204: 348-57.

Feldmann, M., F. M. Brennan, and R. N. Maini. 1996. 'Role of cytokines in rheumatoid arthritis', *Annu Rev Immunol*, 14: 397-440.

Fleury, O. M., M. A. McAleer, C. Feuillie, C. Formosa-Dague, E. Sansevere, D. E. Bennett, A. M. Towell, W. H. I. McLean, S. Kezic, D. A. Robinson, P. G. Fallon, T. J. Foster, Y. F. Dufrene, A. D. Irvine, and J. A. Geoghegan. 2017. 'Clumping Factor B Promotes Adherence of Staphylococcus aureus to Corneocytes in Atopic Dermatitis', *Infect Immun*, 85.

Foster, T. J. 2019. 'Surface Proteins of Staphylococcus aureus', *Microbiol Spectr*, 7.

Foster, T. J., J. A. Geoghegan, V. K. Ganesh, and M. Hook. 2014. 'Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus', *Nat Rev Microbiol*, 12: 49-62.

Fournier, B. 2012. 'The function of TLR2 during staphylococcal diseases', *Front Cell Infect Microbiol*, 2: 167.

Fournier, B., and D. J. Philpott. 2005. 'Recognition of Staphylococcus aureus by the innate immune system', *Clin Microbiol Rev*, 18: 521-40.

Fraser, J. D. 2011. 'Clarifying the mechanism of superantigen toxicity', *PLoS Biol*, 9: e1001145.

Fukuda, A., S. Matsuyama, T. Hara, J. Nakayama, H. Nagasawa, and H. Tokuda. 2002. 'Aminoacylation of the N-terminal cysteine is essential for Lol-dependent release of lipoproteins from membranes but does not depend on lipoprotein sorting signals', *J Biol Chem*, 277: 43512-8.

Galloway, J. B., K. L. Hyrich, L. K. Mercer, W. G. Dixon, A. P. Ustianowski, M. Helbert, K. D. Watson, M. Lunt, D. P. Symmons, and B. S. R. Biologics Register. 2011. 'Risk of septic arthritis in patients with rheumatoid arthritis and the effect of anti-TNF therapy: results from the British Society for Rheumatology Biologics Register', *Ann Rheum Dis*, 70: 1810-4.

Gan, K., S. D. Gupta, K. Sankaran, M. B. Schmid, and H. C. Wu. 1993. 'Isolation and characterization of a temperature-sensitive mutant of Salmonella typhimurium defective in prolipoprotein modification', *J Biol Chem*, 268: 16544-50.

Gardiner, J. H. th, G. Komazin, M. Matsuo, K. Cole, F. Gotz, and T. C. Meredith. 2020. 'Lipoprotein N-Acylation in Staphylococcus aureus Is Catalyzed by a Two-Component Acyl Transferase System', *mBio*, 11.

Giesbrecht, P., T. Kersten, H. Maidhof, and J. Wecke. 1998. 'Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin', *Microbiol Mol Biol Rev*, 62: 1371-414.

Gjertsson, I., O. H. Hultgren, M. Stenson, R. Holmdahl, and A. Tarkowski. 2000. 'Are B lymphocytes of importance in severe Staphylococcus aureus infections?', *Infect Immun*, 68: 2431-4.

Goldenberg, D. L. 1998. 'Septic arthritis', *Lancet*, 351: 197-202.

Goldenberg, D. L., and J. I. Reed. 1985. 'Bacterial arthritis', *N Engl J Med*, 312: 764-71.

Graf, A., R. J. Lewis, S. Fuchs, M. Pagels, S. Engelmann, K. Riedel, and J. Pane-Farre. 2018. 'The hidden lipoproteome of Staphylococcus aureus', *Int J Med Microbiol*, 308: 569-81.

Grice, E. A., and J. A. Segre. 2011. 'The skin microbiome', *Nat Rev Microbiol*, 9: 244-53.

Grundmann, H., M. Aires-de-Sousa, J. Boyce, and E. Tiemersma. 2006. 'Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat', *Lancet*, 368: 874-85.

Guerra, F. E., T. R. Borgogna, D. M. Patel, E. W. Sward, and J. M. Voyich. 2017. 'Epic Immune Battles of History: Neutrophils vs. Staphylococcus aureus', *Front Cell Infect Microbiol*, 7: 286.

Guidry, A. J., S. P. Oliver, K. E. Squiggins, E. F. Erbe, H. H. Dowlen, C. N. Hambleton, and L. M. Berning. 1991. 'Effect of anticapsular antibodies on neutrophil phagocytosis of Staphylococcus aureus', *J Dairy Sci*, 74: 3360-9.

Gupta, S. D., and H. C. Wu. 1991. 'Identification and subcellular localization of apolipoprotein N-acyltransferase in Escherichia coli', *FEMS Microbiol Lett*, 62: 37-41.

Haley, K. P., and E. P. Skaar. 2012. 'A battle for iron: host sequestration and Staphylococcus aureus acquisition', *Microbes Infect*, 14: 217-27.

Hamilton, A., C. Robinson, I. C. Sutcliffe, J. Slater, D. J. Maskell, N. Davis-Poynter, K. Smith, A. Waller, and D. J. Harrington. 2006. 'Mutation of the maturase lipoprotein attenuates the virulence of Streptococcus equi to a greater extent than does loss of general lipoprotein lipidation', *Infect Immun*, 74: 6907-19.

Hammer, N. D., and E. P. Skaar. 2011. 'Molecular mechanisms of Staphylococcus aureus iron acquisition', *Annu Rev Microbiol*, 65: 129-47.

Handler, M. Z., and R. A. Schwartz. 2014. 'Staphylococcal scalded skin syndrome: diagnosis and management in children and adults', *J Eur Acad Dermatol Venereol*, 28: 1418-23.

Hanks, T. S., M. Liu, M. J. McClure, and B. Lei. 2005. 'ABC transporter FtsABCD of Streptococcus pyogenes mediates uptake of ferric ferrichrome', *BMC Microbiol*, 5: 62.

Hantke, K., and V. Braun. 1973. 'Covalent binding of lipid to protein. Diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the Escherichia coli outer membrane', *Eur J Biochem*, 34: 284-96.

Hanzelmann, D., H. S. Joo, M. Franz-Wachtel, T. Hertlein, S. Stevanovic, B. Macek, C. Wolz, F. Gotz, M. Otto, D. Kretschmer, and A. Peschel. 2016. 'Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants', *Nat Commun*, 7: 12304.

Hashimoto, M., K. Tawaratsumida, H. Kariya, K. Aoyama, T. Tamura, and Y. Suda. 2006. 'Lipoprotein is a predominant Toll-like receptor 2 ligand in Staphylococcus aureus cell wall components', *Int Immunol*, 18: 355-62.

Hashimoto, M., K. Tawaratsumida, H. Kariya, A. Kiyohara, Y. Suda, F. Krikae, T. Kirikae, and F. Gotz. 2006. 'Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in Staphylococcus aureus', *J Immunol*, 177: 3162-9.

Hayashi, S., S. Y. Chang, S. Chang, C. Z. Giam, and H. C. Wu. 1985. 'Modification and processing of internalized signal sequences of

prolipoprotein in Escherichia coli and in Bacillus subtilis', *J Biol Chem*, 260: 5753-9.

Hayashi, S., and H. C. Wu. 1990. 'Lipoproteins in bacteria', *J Bioenerg Biomembr*, 22: 451-71.

Heinrichs, J. H., L. E. Gatlin, C. Kunsch, G. H. Choi, and M. S. Hanson. 1999. 'Identification and characterization of SirA, an iron-regulated protein from Staphylococcus aureus', *J Bacteriol*, 181: 1436-43.

Hempel, K., F. A. Herbst, M. Moche, M. Hecker, and D. Becher. 2011. 'Quantitative proteomic view on secreted, cell surface-associated, and cytoplasmic proteins of the methicillin-resistant human pathogen Staphylococcus aureus under iron-limited conditions', *J Proteome Res*, 10: 1657-66.

Hendrickx, A. P., J. M. Budzik, S. Y. Oh, and O. Schneewind. 2011. 'Architects at the bacterial surface - sortases and the assembly of pili with isopeptide bonds', *Nat Rev Microbiol*, 9: 166-76.

Henneke, P., S. Dramsi, G. Mancuso, K. Chraibi, E. Pellegrini, C. Theilacker, J. Hubner, S. Santos-Sierra, G. Teti, D. T. Golenbock, C. Poyart, and P. Trieu-Cuot. 2008. 'Lipoproteins are critical TLR2 activating toxins in group B streptococcal sepsis', *J Immunol*, 180: 6149-58.

Herbert, S., A. K. Ziebandt, K. Ohlsen, T. Schafer, M. Hecker, D. Albrecht, R. Novick, and F. Gotz. 2010. 'Repair of global regulators in Staphylococcus aureus 8325 and comparative analysis with other clinical isolates', *Infect Immun*, 78: 2877-89.

Hilmi, D., M. Parcina, D. Stollewerk, J. Ostrop, M. Josten, A. Meilaender, U. Zaehringer, T. A. Wichelhaus, G. Bierbaum, K. Heeg, C. Wolz, and I. Bekeredjian-Ding. 2014. 'Heterogeneity of host TLR2 stimulation by Staphylocoocus aureus isolates', *PLoS One*, 9: e96416.

Hoffmann, P., S. Heinle, U. F. Schade, H. Loppnow, A. J. Ulmer, H. D. Flad, G. Jung, and W. G. Bessler. 1988. 'Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues', *Immunobiology*, 177: 158-70.

Hong, S. W., E. B. Choi, T. K. Min, J. H. Kim, M. H. Kim, S. G. Jeon, B. J. Lee, Y. S. Gho, Y. K. Jee, B. Y. Pyun, and Y. K. Kim. 2014. 'An important role of alpha-hemolysin in extracellular vesicles on the development of atopic dermatitis induced by Staphylococcus aureus', *PLoS One*, 9: e100499.

Huang, Z., X. Yi, Y. Chen, X. Hou, X. Wang, P. Zhu, K. Zhao, S. Wu, N. Fu, and B. Liu. 2017. 'Pretreatment of Pam3CSK4 attenuates inflammatory responses caused by systemic infection of methicillin-resistant Staphylococcus aureus in mice', *Biomed Pharmacother*, 95: 1684-92.

Hultgren, O., H. P. Eugster, J. D. Sedgwick, H. Korner, and A. Tarkowski. 1998. 'TNF/lymphotoxin-alpha double-mutant mice resist septic arthritis but display increased mortality in response to Staphylococcus aureus', *J Immunol*, 161: 5937-42.

Hultgren, O. H., L. Svensson, and A. Tarkowski. 2002. 'Critical role of signaling through IL-1 receptor for development of arthritis and sepsis during Staphylococcus aureus infection', *J Immunol*, 168: 5207-12.

Hussain, M., S. Ichihara, and S. Mizushima. 1982. 'Mechanism of signal peptide cleavage in the biosynthesis of the major lipoprotein of the Escherichia coli outer membrane', *J Biol Chem*, 257: 5177-82.

Hutchings, M. I., T. Palmer, D. J. Harrington, and I. C. Sutcliffe. 2009. 'Lipoprotein biogenesis in Gram-positive bacteria: knowing when to hold 'em, knowing when to fold 'em', *Trends Microbiol*, 17: 13-21.

Janulczyk, R., J. Pallon, and L. Bjorck. 1999. 'Identification and characterization of a Streptococcus pyogenes ABC transporter with multiple specificity for metal cations', *Mol Microbiol*, 34: 596-606.

Jarneborn, A., M. Mohammad, C. Engdahl, Z. Hu, M. Na, A. Ali, and T. Jin. 2020. 'Tofacitinib treatment aggravates Staphylococcus aureus septic arthritis, but attenuates sepsis and enterotoxin induced shock in mice', *Sci Rep*, 10: 10891.

Jensen, K. 1958. 'A normally occurring Staphylococcus antibody in human serum', *APMIS*, 115: 533-9; discussion 40-1.

Jin, M. S., S. E. Kim, J. Y. Heo, M. E. Lee, H. M. Kim, S. G. Paik, H. Lee, and J. O. Lee. 2007. 'Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide', *Cell*, 130: 1071-82.

Jin, T., M. Mohammad, Z. Hu, Y. Fei, E. R. B. Moore, R. Pullerits, and A. Ali. 2019. 'A novel mouse model for septic arthritis induced by Pseudomonas aeruginosa', *Sci Rep*, 9: 16868.

Jin, T., Y. L. Zhu, J. Li, J. Shi, X. Q. He, J. Ding, and Y. Q. Xu. 2013. 'Staphylococcal protein A, Panton-Valentine leukocidin and coagulase aggravate the bone loss and bone destruction in osteomyelitis', *Cell Physiol Biochem*, 32: 322-33.

Josefsson, E., O. Hartford, L. O'Brien, J. M. Patti, and T. Foster. 2001. 'Protection against experimental Staphylococcus aureus arthritis by vaccination with clumping factor A, a novel virulence determinant', *J Infect Dis*, 184: 1572-80.

Kaandorp, C. J., H. J. Dinant, M. A. van de Laar, H. J. Moens, A. P. Prins, and B. A. Dijkmans. 1997. 'Incidence and sources of native and prosthetic joint infection: a community based prospective survey', *Ann Rheum Dis*, 56: 470-5.

Kaandorp, C. J., D. Van Schaardenburg, P. Krijnen, J. D. Habbema, and M. A. van de Laar. 1995. 'Risk factors for septic arthritis in patients with joint disease. A prospective study', *Arthritis Rheum*, 38: 1819-25.

Kang, H. J., J. M. Ha, H. S. Kim, H. Lee, K. Kurokawa, and B. L. Lee. 2011. 'The role of phagocytosis in IL-8 production by human monocytes in response to lipoproteins on Staphylococcus aureus', *Biochem Biophys Res Commun*, 406: 449-53.

Kang, J. Y., X. Nan, M. S. Jin, S. J. Youn, Y. H. Ryu, S. Mah, S. H. Han, H. Lee, S. G. Paik, and J. O. Lee. 2009. 'Recognition of lipopeptide

patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer', *Immunity*, 31: 873-84.

Kaplan, J. B., E. A. Izano, P. Gopal, M. T. Karwacki, S. Kim, J. L. Bose, K. W. Bayles, and A. R. Horswill. 2012. 'Low levels of beta-lactam antibiotics induce extracellular DNA release and biofilm formation in Staphylococcus aureus', *mBio*, 3: e00198-12.

Kebaier, C., R. R. Chamberland, I. C. Allen, X. Gao, P. M. Broglie, J. D. Hall, C. Jania, C. M. Doerschuk, S. L. Tilley, and J. A. Duncan. 2012. 'Staphylococcus aureus alpha-hemolysin mediates virulence in a murine model of severe pneumonia through activation of the NLRP3 inflammasome', *J Infect Dis*, 205: 807-17.

Kennedy, A. D., J. Bubeck Wardenburg, D. J. Gardner, D. Long, A. R. Whitney, K. R. Braughton, O. Schneewind, and F. R. DeLeo. 2010. 'Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model', *J Infect Dis*, 202: 1050-8.

Kernodle, D. S., P. A. McGraw, N. L. Barg, B. E. Menzies, R. K. Voladri, and S. Harshman. 1995. 'Growth of Staphylococcus aureus with nafcillin in vitro induces alpha-toxin production and increases the lethal activity of sterile broth filtrates in a murine model', *J Infect Dis*, 172: 410-9.

Keshav, S., P. Chung, G. Milon, and S. Gordon. 1991. 'Lysozyme is an inducible marker of macrophage activation in murine tissues as demonstrated by in situ hybridization', *J Exp Med*, 174: 1049-58.

Kierdorf, K., M. Prinz, F. Geissmann, and E. Gomez Perdiguero. 2015. 'Development and function of tissue resident macrophages in mice', *Semin Immunol*, 27: 369-78.

Kim, H. Y., A. R. Kim, H. S. Seo, J. E. Baik, K. B. Ahn, C. H. Yun, and S. H. Han. 2018. 'Lipoproteins in Streptococcus gordonii are critical in the infection and inflammatory responses', *Mol Immunol*, 101: 574-84.

Kim, N. J., K. B. Ahn, J. H. Jeon, C. H. Yun, B. B. Finlay, and S. H. Han. 2015. 'Lipoprotein in the cell wall of Staphylococcus aureus is a major inducer of nitric oxide production in murine macrophages', *Mol Immunol*, 65: 17-24.

Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey, S. K. Fridkin, and Mrsa Investigators Active Bacterial Core surveillance. 2007. 'Invasive methicillin-resistant Staphylococcus aureus infections in the United States', *JAMA*, 298: 1763-71.

Kobayashi, S. D., N. Malachowa, and F. R. DeLeo. 2015. 'Pathogenesis of Staphylococcus aureus abscesses', *Am J Pathol*, 185: 1518-27.

Kobayashi, S. D., N. Malachowa, A. R. Whitney, K. R. Braughton, D. J. Gardner, D. Long, J. Bubeck Wardenburg, O. Schneewind, M. Otto, and F. R. Deleo. 2011. 'Comparative analysis of USA300 virulence

determinants in a rabbit model of skin and soft tissue infection', *J Infect Dis*, 204: 937-41.
Kolaczkowska, E., and P. Kubes. 2013. 'Neutrophil recruitment and function in health and inflammation', *Nat Rev Immunol*, 13: 159-75.
Kong, C., H. M. Neoh, and S. Nathan. 2016. 'Targeting Staphylococcus aureus Toxins: A Potential form of Anti-Virulence Therapy', *Toxins (Basel)*, 8.
Kovacs-Simon, A., R. W. Titball, and S. L. Michell. 2011. 'Lipoproteins of bacterial pathogens', *Infect Immun*, 79: 548-61.
Koymans, K. J., L. J. Feitsma, T. H. Brondijk, P. C. Aerts, E. Lukkien, P. Lossl, K. P. van Kessel, C. J. de Haas, J. A. van Strijp, and E. G. Huizinga. 2015. 'Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3)', *Proc Natl Acad Sci U S A*, 112: 11018-23.
Kreutz, M., U. Ackermann, S. Hauschildt, S. W. Krause, D. Riedel, W. Bessler, and R. Andreesen. 1997. 'A comparative analysis of cytokine production and tolerance induction by bacterial lipopeptides, lipopolysaccharides and Staphyloccous aureus in human monocytes', *Immunology*, 92: 396-401.
Krishna, S., and L. S. Miller. 2012a. 'Host-pathogen interactions between the skin and Staphylococcus aureus', *Curr Opin Microbiol*, 15: 28-35.
———. 2012b. 'Innate and adaptive immune responses against Staphylococcus aureus skin infections', *Semin Immunopathol*, 34: 261-80.
Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu. 2001. 'Whole genome sequencing of meticillin-resistant Staphylococcus aureus', *Lancet*, 357: 1225-40.
Kurokawa, K., M. S. Kim, R. Ichikawa, K. H. Ryu, N. Dohmae, H. Nakayama, and B. L. Lee. 2012. 'Environment-mediated accumulation of diacyl lipoproteins over their triacyl counterparts in Staphylococcus aureus', *J Bacteriol*, 194: 3299-306.
Kurokawa, K., H. Lee, K. B. Roh, M. Asanuma, Y. S. Kim, H. Nakayama, A. Shiratsuchi, Y. Choi, O. Takeuchi, H. J. Kang, N. Dohmae, Y. Nakanishi, S. Akira, K. Sekimizu, and B. L. Lee. 2009. 'The Triacylated ATP Binding Cluster Transporter Substrate-binding Lipoprotein of Staphylococcus aureus Functions as a Native Ligand for Toll-like Receptor 2', *J Biol Chem*, 284: 8406-11.
Kurokawa, K., K. H. Ryu, R. Ichikawa, A. Masuda, M. S. Kim, H. Lee, J. H. Chae, T. Shimizu, T. Saitoh, K. Kuwano, S. Akira, N. Dohmae, H.

Nakayama, and B. L. Lee. 2012. 'Novel bacterial lipoprotein structures conserved in low-GC content gram-positive bacteria are recognized by Toll-like receptor 2', *J Biol Chem*, 287: 13170-81.

Kusunoki, T., E. Hailman, T. S. Juan, H. S. Lichenstein, and S. D. Wright. 1995. 'Molecules from Staphylococcus aureus that bind CD14 and stimulate innate immune responses', *J Exp Med*, 182: 1673-82.

Kwiecinski, J., G. Jacobsson, M. Karlsson, X. Zhu, W. Wang, T. Bremell, E. Josefsson, and T. Jin. 2013. 'Staphylokinase promotes the establishment of Staphylococcus aureus skin infections while decreasing disease severity', *J Infect Dis*, 208: 990-9.

Kwiecinski, J., T. Jin, and E. Josefsson. 2014. 'Surface proteins of Staphylococcus aureus play an important role in experimental skin infection', *APMIS*, 122: 1240-50.

Kwiecinski, J., E. Josefsson, J. Mitchell, J. Higgins, M. Magnusson, T. Foster, T. Jin, and M. Bokarewa. 2010. 'Activation of plasminogen by staphylokinase reduces the severity of Staphylococcus aureus systemic infection', *J Infect Dis*, 202: 1041-9.

Lacey, K. A., J. M. Leech, S. J. Lalor, N. McCormack, J. A. Geoghegan, and R. M. McLoughlin. 2017. 'The Staphylococcus aureus Cell Wall-Anchored Protein Clumping Factor A Is an Important T Cell Antigen', *Infect Immun*, 85.

Lacey, K. A., M. E. Mulcahy, A. M. Towell, J. A. Geoghegan, and R. M. McLoughlin. 2019. 'Clumping factor B is an important virulence factor during Staphylococcus aureus skin infection and a promising vaccine target', *PLoS Pathog*, 15: e1007713.

Lappin, E., and A. J. Ferguson. 2009. 'Gram-positive toxic shock syndromes', *Lancet Infect Dis*, 9: 281-90.

Lee, C. C., A. M. Avalos, and H. L. Ploegh. 2012. 'Accessory molecules for Toll-like receptors and their function', *Nat Rev Immunol*, 12: 168-79.

Lee, J., G. Cacalano, T. Camerato, K. Toy, M. W. Moore, and W. I. Wood. 1995. 'Chemokine binding and activities mediated by the mouse IL-8 receptor', *J Immunol*, 155: 2158-64.

Lei, B., M. Liu, J. M. Voyich, C. I. Prater, S. V. Kala, F. R. DeLeo, and J. M. Musser. 2003. 'Identification and characterization of HtsA, a second heme-binding protein made by Streptococcus pyogenes', *Infect Immun*, 71: 5962-9.

Leskela, S., E. Wahlstrom, V. P. Kontinen, and M. Sarvas. 1999. 'Lipid modification of prelipoproteins is dispensable for growth but essential for efficient protein secretion in Bacillus subtilis: characterization of the Lgt gene', *Mol Microbiol*, 31: 1075-85.

Leung, A. K. C., B. Barankin, and K. F. Leong. 2018. 'Staphylococcal-scalded skin syndrome: evaluation, diagnosis, and management', *World J Pediatr*, 14: 116-20.

Lin, S. C., Y. C. Lo, and H. Wu. 2010. 'Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling', *Nature*, 465: 885-90.
Liu, Z. Q., G. M. Deng, S. Foster, and A. Tarkowski. 2001. 'Staphylococcal peptidoglycans induce arthritis', *Arthritis Res*, 3: 375-80.
Loder, F., B. Mutschler, R. J. Ray, C. J. Paige, P. Sideras, R. Torres, M. C. Lamers, and R. Carsetti. 1999. 'B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals', *J Exp Med*, 190: 75-89.
Low, D. E. 2013. 'Toxic shock syndrome: major advances in pathogenesis, but not treatment', *Crit Care Clin*, 29: 651-75.
Lowy, F. D. 1998. 'Staphylococcus aureus infections', *N Engl J Med*, 339: 520-32.
Machata, S., S. Tchatalbachev, W. Mohamed, L. Jansch, T. Hain, and T. Chakraborty. 2008. 'Lipoproteins of Listeria monocytogenes are critical for virulence and TLR2-mediated immune activation', *J Immunol*, 181: 2028-35.
Madani, A., K. Garakani, and M. R. K. Mofrad. 2017. 'Molecular mechanics of Staphylococcus aureus adhesin, CNA, and the inhibition of bacterial adhesion by stretching collagen', *PLoS One*, 12: e0179601.
Malachowa, N., S. D. Kobayashi, A. R. Porter, K. R. Braughton, D. P. Scott, D. J. Gardner, D. M. Missiakas, O. Schneewind, and F. R. DeLeo. 2016. 'Contribution of Staphylococcus aureus Coagulases and Clumping Factor A to Abscess Formation in a Rabbit Model of Skin and Soft Tissue Infection', *PLoS One*, 11: e0158293.
Margaretten, M. E., J. Kohlwes, D. Moore, and S. Bent. 2007. 'Does this adult patient have septic arthritis?', *JAMA*, 297: 1478-88.
Mathews, C. J., V. C. Weston, A. Jones, M. Field, and G. Coakley. 2010. 'Bacterial septic arthritis in adults', *Lancet*, 375: 846-55.
Mazmanian, S. K., H. Ton-That, and O. Schneewind. 2001. 'Sortase-catalysed anchoring of surface proteins to the cell wall of Staphylococcus aureus', *Mol Microbiol*, 40: 1049-57.
Mazmanian, S. K., H. Ton-That, K. Su, and O. Schneewind. 2002. 'An iron-regulated sortase anchors a class of surface protein during Staphylococcus aureus pathogenesis', *Proc Natl Acad Sci U S A*, 99: 2293-8.
McAdow, M., D. M. Missiakas, and O. Schneewind. 2012. 'Staphylococcus aureus secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections', *J Innate Immun*, 4: 141-8.
McCaig, L. F., L. C. McDonald, S. Mandal, and D. B. Jernigan. 2006. 'Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care', *Emerg Infect Dis*, 12: 1715-23.
McCarthy, A. J., and J. A. Lindsay. 2010. 'Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated:

implications for vaccine design and host-pathogen interactions', *BMC Microbiol*, 10: 173.

McCormick, J. K., T. J. Tripp, A. S. Llera, E. J. Sundberg, M. M. Dinges, R. A. Mariuzza, and P. M. Schlievert. 2003. 'Functional analysis of the TCR binding domain of toxic shock syndrome toxin-1 predicts further diversity in MHC class II/superantigen/TCR ternary complexes', *J Immunol*, 171: 1385-92.

Mestas, J., and C. C. Hughes. 2004. 'Of mice and not men: differences between mouse and human immunology', *J Immunol*, 172: 2731-8.

Miller, L. S., and J. S. Cho. 2011. 'Immunity against Staphylococcus aureus cutaneous infections', *Nat Rev Immunol*, 11: 505-18.

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

Mohamed, N., Y. Timofeyeva, D. Jamrozy, E. Rojas, L. Hao, N. C. Silmon de Monerri, J. Hawkins, G. Singh, B. Cai, P. Liberator, S. Sebastian, R. G. K. Donald, I. L. Scully, C. H. Jones, C. B. Creech, I. Thomsen, J. Parkhill, S. J. Peacock, K. U. Jansen, M. T. G. Holden, and A. S. Anderson. 2019. 'Molecular epidemiology and expression of capsular polysaccharides in Staphylococcus aureus clinical isolates in the United States', *PLoS One*, 14: e0208356.

Mohammad, M., M. Na, A. Welin, M. N. Svensson, A. Ali, T. Jin, and R. Pullerits. 2016. 'RAGE Deficiency Impairs Bacterial Clearance in Murine Staphylococcal Sepsis, but Has No Significant Impact on Staphylococcal Septic Arthritis', *PLoS One*, 11: e0167287.

Mohammad, M., M. T. Nguyen, C. Engdahl, M. Na, A. Jarneborn, Z. Hu, A. Karlsson, R. Pullerits, A. Ali, F. Gotz, and T. Jin. 2019. 'The YIN and YANG of lipoproteins in developing and preventing infectious arthritis by Staphylococcus aureus', *PLoS Pathog*, 15: e1007877.

Mohammad, Majd, Zhicheng Hu, Abukar Ali, Pradeep Kumar Kopparapu, Manli Na, Anders Jarneborn, Mariana do Nascimento Stroparo, Minh-Thu Nguyen, Anna Karlsson, Friedrich Götz, Rille Pullerits, and Tao Jin. 2020. 'The role of Staphylococcus aureus lipoproteins in hematogenous septic arthritis', *Scientific Reports*, 10: 7936.

Mohiman, N., M. Argentini, S. M. Batt, D. Cornu, M. Masi, L. Eggeling, G. Besra, and N. Bayan. 2012. 'The ppm operon is essential for acylation and glycosylation of lipoproteins in Corynebacterium glutamicum', *PLoS One*, 7: e46225.

Molkanen, T., J. Tyynela, J. Helin, N. Kalkkinen, and P. Kuusela. 2002. 'Enhanced activation of bound plasminogen on Staphylococcus aureus by staphylokinase', *FEBS Lett*, 517: 72-8.

Molne, L., M. Verdrengh, and A. Tarkowski. 2000. 'Role of neutrophil leukocytes in cutaneous infection caused by Staphylococcus aureus', *Infect Immun*, 68: 6162-7.

Montgomery, C. P., M. Daniels, F. Zhao, M. L. Alegre, A. S. Chong, and R. S. Daum. 2014. 'Protective immunity against recurrent Staphylococcus aureus skin infection requires antibody and interleukin-17A', *Infect Immun*, 82: 2125-34.

Morath, S., A. Geyer, and T. Hartung. 2001. 'Structure-function relationship of cytokine induction by lipoteichoic acid from Staphylococcus aureus', *J Exp Med*, 193: 393-7.

Morath, S., A. Stadelmaier, A. Geyer, R. R. Schmidt, and T. Hartung. 2002. 'Synthetic lipoteichoic acid from Staphylococcus aureus is a potent stimulus of cytokine release', *J Exp Med*, 195: 1635-40.

Morrissey, J. A., A. Cockayne, P. J. Hill, and P. Williams. 2000. 'Molecular cloning and analysis of a putative siderophore ABC transporter from Staphylococcus aureus', *Infect Immun*, 68: 6281-8.

Muller-Anstett, M. A., P. Muller, T. Albrecht, M. Nega, J. Wagener, Q. Gao, S. Kaesler, M. Schaller, T. Biedermann, and F. Gotz. 2010. 'Staphylococcal peptidoglycan co-localizes with Nod2 and TLR2 and activates innate immune response via both receptors in primary murine keratinocytes', *PLoS One*, 5: e13153.

Muller, P., M. Muller-Anstett, J. Wagener, Q. Gao, S. Kaesler, M. Schaller, T. Biedermann, and F. Gotz. 2010. 'The Staphylococcus aureus lipoprotein SitC colocalizes with Toll-like receptor 2 (TLR2) in murine keratinocytes and elicits intracellular TLR2 accumulation', *Infect Immun*, 78: 4243-50.

Muzio, M., G. Natoli, S. Saccani, M. Levrero, and A. Mantovani. 1998. 'The human toll signaling pathway: divergence of nuclear factor kappaB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6)', *J Exp Med*, 187: 2097-101.

Na, M., A. Jarneborn, A. Ali, A. Welin, M. Magnusson, A. Stokowska, M. Pekna, and T. Jin. 2016. 'Deficiency of the Complement Component 3 but Not Factor B Aggravates Staphylococcus aureus Septic Arthritis in Mice', *Infect Immun*, 84: 930-39.

Na, M., W. Wang, Y. Fei, E. Josefsson, A. Ali, and T. Jin. 2017. 'Both anti-TNF and CTLA4 Ig treatments attenuate the disease severity of staphylococcal dermatitis in mice', *PLoS One*, 12: e0173492.

Nade, S. 2003. 'Septic arthritis', *Best Pract Res Clin Rheumatol*, 17: 183-200.

Nakayama, H., K. Kurokawa, and B. L. Lee. 2012. 'Lipoproteins in bacteria: structures and biosynthetic pathways', *FEBS J*, 279: 4247-68.

Nanra, J. S., S. M. Buitrago, S. Crawford, J. Ng, P. S. Fink, J. Hawkins, I. L. Scully, L. K. McNeil, J. M. Aste-Amezaga, D. Cooper, K. U. Jansen, and A. S. Anderson. 2013. 'Capsular polysaccharides are an important immune evasion mechanism for Staphylococcus aureus', *Hum Vaccin Immunother*, 9: 480-7.

Navarre, W. W., S. Daefler, and O. Schneewind. 1996. 'Cell wall sorting of lipoproteins in Staphylococcus aureus', *J Bacteriol*, 178: 441-6.

Nguyen, M. T., M. Deplanche, M. Nega, Y. Le Loir, L. Peisl, F. Gotz, and N. Berkova. 2016. 'Staphylococcus aureus Lpl Lipoproteins Delay G2/M Phase Transition in HeLa Cells', *Front Cell Infect Microbiol*, 6: 201.

Nguyen, M. T., and F. Gotz. 2016. 'Lipoproteins of Gram-Positive Bacteria: Key Players in the Immune Response and Virulence', *Microbiol Mol Biol Rev*, 80: 891-903.

Nguyen, M. T., B. Kraft, W. Yu, D. D. Demircioglu, T. Hertlein, M. Burian, M. Schmaler, K. Boller, I. Bekeredjian-Ding, K. Ohlsen, B. Schittek, and F. Gotz. 2015. 'The nuSaalpha Specific Lipoprotein Like Cluster (lpl) of S. aureus USA300 Contributes to Immune Stimulation and Invasion in Human Cells', *PLoS Pathog*, 11: e1004984.

Nguyen, M. T., L. Peisl, F. Barletta, A. Luqman, and F. Gotz. 2018. 'Toll-Like Receptor 2 and Lipoprotein-Like Lipoproteins Enhance Staphylococcus aureus Invasion in Epithelial Cells', *Infect Immun*, 86.

Nguyen, M. T., J. Uebele, N. Kumari, H. Nakayama, L. Peter, O. Ticha, A. K. Woischnig, M. Schmaler, N. Khanna, N. Dohmae, B. L. Lee, I. Bekeredjian-Ding, and F. Gotz. 2017. 'Lipid moieties on lipoproteins of commensal and non-commensal staphylococci induce differential immune responses', *Nat Commun*, 8: 2246.

Ni Eidhin, D., S. Perkins, P. Francois, P. Vaudaux, M. Hook, and T. J. Foster. 1998. 'Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of Staphylococcus aureus', *Mol Microbiol*, 30: 245-57.

Nilsson, I. M., T. Bremell, C. Ryden, A. L. Cheung, and A. Tarkowski. 1996. 'Role of the staphylococcal accessory gene regulator (sar) in septic arthritis', *Infect Immun*, 64: 4438-43.

Nilsson, I. M., O. Hartford, T. Foster, and A. Tarkowski. 1999. 'Alpha-toxin and gamma-toxin jointly promote Staphylococcus aureus virulence in murine septic arthritis', *Infect Immun*, 67: 1045-9.

Nilsson, I. M., J. C. Lee, T. Bremell, C. Ryden, and A. Tarkowski. 1997. 'The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis', *Infect Immun*, 65: 4216-21.

Norgard, M. V., L. L. Arndt, D. R. Akins, L. L. Curetty, D. A. Harrich, and J. D. Radolf. 1996. 'Activation of human monocytic cells by Treponema pallidum and Borrelia burgdorferi lipoproteins and synthetic lipopeptides proceeds via a pathway distinct from that of lipopolysaccharide but involves the transcriptional activator NF-kappa B', *Infect Immun*, 64: 3845-52.

O'Riordan, K., and J. C. Lee. 2004. 'Staphylococcus aureus capsular polysaccharides', *Clin Microbiol Rev*, 17: 218-34.

Okugawa, S., M. Moayeri, A. P. Pomerantsev, I. Sastalla, D. Crown, P. K. Gupta, and S. H. Leppla. 2012. 'Lipoprotein biosynthesis by prolipoprotein diacylglyceryl transferase is required for efficient spore

germination and full virulence of Bacillus anthracis', *Mol Microbiol*, 83: 96-109.
Oliveira, D., A. Borges, and M. Simoes. 2018. 'Staphylococcus aureus Toxins and Their Molecular Activity in Infectious Diseases', *Toxins (Basel)*, 10.
Otto, M. 2004. 'Quorum-sensing control in Staphylococci -- a target for antimicrobial drug therapy?', *FEMS Microbiol Lett*, 241: 135-41.
Palmqvist, N., T. Foster, J. R. Fitzgerald, E. Josefsson, and A. Tarkowski. 2005. 'Fibronectin-binding proteins and fibrinogen-binding clumping factors play distinct roles in staphylococcal arthritis and systemic inflammation', *J Infect Dis*, 191: 791-8.
Palmqvist, N., T. Foster, A. Tarkowski, and E. Josefsson. 2002. 'Protein A is a virulence factor in Staphylococcus aureus arthritis and septic death', *Microb Pathog*, 33: 239-49.
Patti, J. M., B. L. Allen, M. J. McGavin, and M. Hook. 1994. 'MSCRAMM-mediated adherence of microorganisms to host tissues', *Annu Rev Microbiol*, 48: 585-617.
Patti, J. M., T. Bremell, D. Krajewska-Pietrasik, A. Abdelnour, A. Tarkowski, C. Ryden, and M. Hook. 1994. 'The Staphylococcus aureus collagen adhesin is a virulence determinant in experimental septic arthritis', *Infect Immun*, 62: 152-61.
Pennock, N. D., J. T. White, E. W. Cross, E. E. Cheney, B. A. Tamburini, and R. M. Kedl. 2013. 'T cell responses: naive to memory and everything in between', *Adv Physiol Educ*, 37: 273-83.
Periasamy, S., H. S. Joo, A. C. Duong, T. H. Bach, V. Y. Tan, S. S. Chatterjee, G. Y. Cheung, and M. Otto. 2012. 'How Staphylococcus aureus biofilms develop their characteristic structure', *Proc Natl Acad Sci U S A*, 109: 1281-6.
Peschel, A., and M. Otto. 2013. 'Phenol-soluble modulins and staphylococcal infection', *Nat Rev Microbiol*, 11: 667-73.
Peschel, A., M. Otto, R. W. Jack, H. Kalbacher, G. Jung, and F. Gotz. 1999. 'Inactivation of the dlt operon in Staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides', *J Biol Chem*, 274: 8405-10.
Petit, C. M., J. R. Brown, K. Ingraham, A. P. Bryant, and D. J. Holmes. 2001. 'Lipid modification of prelipoproteins is dispensable for growth in vitro but essential for virulence in Streptococcus pneumoniae', *FEMS Microbiol Lett*, 200: 229-33.
Pietrocola, G., G. Nobile, M. J. Alfeo, T. J. Foster, J. A. Geoghegan, V. De Filippis, and P. Speziale. 2019. 'Fibronectin-binding protein B (FnBPB) from Staphylococcus aureus protects against the antimicrobial activity of histones', *J Biol Chem*, 294: 3588-602.
Pishchany, G., J. R. Sheldon, C. F. Dickson, M. T. Alam, T. D. Read, D. A. Gell, D. E. Heinrichs, and E. P. Skaar. 2014. 'IsdB-dependent

hemoglobin binding is required for acquisition of heme by Staphylococcus aureus', *J Infect Dis*, 209: 1764-72.

Powers, M. E., H. K. Kim, Y. Wang, and J. Bubeck Wardenburg. 2012. 'ADAM10 mediates vascular injury induced by Staphylococcus aureus alpha-hemolysin', *J Infect Dis*, 206: 352-6.

Proctor, R. A. 2012. 'Is there a future for a Staphylococcus aureus vaccine?', *Vaccine*, 30: 2921-7.

Qin, L., J. W. McCausland, G. Y. Cheung, and M. Otto. 2016. 'PSM-Mec-A Virulence Determinant that Connects Transcriptional Regulation, Virulence, and Antibiotic Resistance in Staphylococci', *Front Microbiol*, 7: 1293.

Rasigade, J. P., S. Trouillet-Assant, T. Ferry, B. A. Diep, A. Sapin, Y. Lhoste, J. Ranfaing, C. Badiou, Y. Benito, M. Bes, F. Couzon, S. Tigaud, G. Lina, J. Etienne, F. Vandenesch, and F. Laurent. 2013. 'PSMs of hypervirulent Staphylococcus aureus act as intracellular toxins that kill infected osteoblasts', *PLoS One*, 8: e63176.

Rawadi, G., J. Garcia, B. Lemercier, and S. Roman-Roman. 1999. 'Signal transduction pathways involved in the activation of NF-kappa B, AP-1, and c-fos by Mycoplasma fermentans membrane lipoproteins in macrophages', *J Immunol*, 162: 2193-203.

Reglier-Poupet, H., C. Frehel, I. Dubail, J. L. Beretti, P. Berche, A. Charbit, and C. Raynaud. 2003. 'Maturation of lipoproteins by type II signal peptidase is required for phagosomal escape of Listeria monocytogenes', *J Biol Chem*, 278: 49469-77.

Reichmann, N. T., and A. Grundling. 2011. 'Location, synthesis and function of glycolipids and polyglycerolphosphate lipoteichoic acid in Gram-positive bacteria of the phylum Firmicutes', *FEMS Microbiol Lett*, 319: 97-105.

Richards, J. B., F. Rivadeneira, M. Inouye, T. M. Pastinen, N. Soranzo, S. G. Wilson, T. Andrew, M. Falchi, R. Gwilliam, K. R. Ahmadi, A. M. Valdes, P. Arp, P. Whittaker, D. J. Verlaan, M. Jhamai, V. Kumanduri, M. Moorhouse, J. B. van Meurs, A. Hofman, H. A. Pols, D. Hart, G. Zhai, B. S. Kato, B. H. Mullin, F. Zhang, P. Deloukas, A. G. Uitterlinden, and T. D. Spector. 2008. 'Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study', *Lancet*, 371: 1505-12.

Robichon, C., D. Vidal-Ingigliardi, and A. P. Pugsley. 2005. 'Depletion of apolipoprotein N-acyltransferase causes mislocalization of outer membrane lipoproteins in Escherichia coli', *J Biol Chem*, 280: 974-83.

Roche, F. M., R. Downer, F. Keane, P. Speziale, P. W. Park, and T. J. Foster. 2004. 'The N-terminal A domain of fibronectin-binding proteins A and B promotes adhesion of Staphylococcus aureus to elastin', *J Biol Chem*, 279: 38433-40.

Rodstrom, K. E., K. Elbing, and K. Lindkvist-Petersson. 2014. 'Structure of the superantigen staphylococcal enterotoxin B in complex with TCR

and peptide-MHC demonstrates absence of TCR-peptide contacts', *J Immunol*, 193: 1998-2004.

Rosendahl Huber, S., J. van Beek, J. de Jonge, W. Luytjes, and D. van Baarle. 2014. 'T cell responses to viral infections - opportunities for Peptide vaccination', *Front Immunol*, 5: 171.

Rosenstein, R., and F. Gotz. 2013. 'What distinguishes highly pathogenic staphylococci from medium- and non-pathogenic?', *Curr Top Microbiol Immunol*, 358: 33-89.

Rutherford, A. I., S. Subesinghe, T. Bharucha, F. Ibrahim, A. Kleymann, and J. B. Galloway. 2016. 'A population study of the reported incidence of native joint septic arthritis in the United Kingdom between 1998 and 2013', *Rheumatology (Oxford)*, 55: 2176-80.

Ryding, U., J. I. Flock, M. Flock, B. Soderquist, and B. Christensson. 1997. 'Expression of collagen-binding protein and types 5 and 8 capsular polysaccharide in clinical isolates of Staphylococcus aureus', *J Infect Dis*, 176: 1096-9.

Saito, S., and A. F. Quadery. 2018. 'Staphylococcus aureus Lipoprotein Induces Skin Inflammation, Accompanied with IFN-gamma-Producing T Cell Accumulation through Dermal Dendritic Cells', *Pathogens*, 7.

Sander, P., M. Rezwan, B. Walker, S. K. Rampini, R. M. Kroppenstedt, S. Ehlers, C. Keller, J. R. Keeble, M. Hagemeier, M. J. Colston, B. Springer, and E. C. Bottger. 2004. 'Lipoprotein processing is required for virulence of Mycobacterium tuberculosis', *Mol Microbiol*, 52: 1543-52.

Sankaran, K., S. D. Gupta, and H. C. Wu. 1995. 'Modification of bacterial lipoproteins', *Methods Enzymol*, 250: 683-97.

Sankaran, K., and H. C. Wu. 1994. 'Lipid modification of bacterial prolipoprotein. Transfer of diacylglyceryl moiety from phosphatidylglycerol', *J Biol Chem*, 269: 19701-6.

Sato, S., H. Sanjo, K. Takeda, J. Ninomiya-Tsuji, M. Yamamoto, T. Kawai, K. Matsumoto, O. Takeuchi, and S. Akira. 2005. 'Essential function for the kinase TAK1 in innate and adaptive immune responses', *Nat Immunol*, 6: 1087-95.

Schaffler, H., D. D. Demircioglu, D. Kuhner, S. Menz, A. Bender, I. B. Autenrieth, P. Bodammer, G. Lamprecht, F. Gotz, and J. S. Frick. 2014. 'NOD2 stimulation by Staphylococcus aureus-derived peptidoglycan is boosted by Toll-like receptor 2 costimulation with lipoproteins in dendritic cells', *Infect Immun*, 82: 4681-8.

Schenk, M., J. T. Belisle, and R. L. Modlin. 2009. 'TLR2 looks at lipoproteins', *Immunity*, 31: 847-9.

Schlatterer, K., C. Beck, D. Hanzelmann, M. Lebtig, B. Fehrenbacher, M. Schaller, P. Ebner, M. Nega, M. Otto, D. Kretschmer, and A. Peschel. 2018. 'The Mechanism behind Bacterial Lipoprotein Release: Phenol-

Soluble Modulins Mediate Toll-Like Receptor 2 Activation via Extracellular Vesicle Release from Staphylococcus aureus', *mBio*, 9.

Schmaler, M., N. J. Jann, F. Ferracin, L. Z. Landolt, L. Biswas, F. Gotz, and R. Landmann. 2009. 'Lipoproteins in Staphylococcus aureus mediate inflammation by TLR2 and iron-dependent growth in vivo', *J Immunol*, 182: 7110-8.

Schmaler, M., N. J. Jann, F. Gotz, and R. Landmann. 2010. 'Staphylococcal lipoproteins and their role in bacterial survival in mice', *Int J Med Microbiol*, 300: 155-60.

Schmollinger, M., I. Fischer, C. Nerz, S. Pinkenburg, F. Gotz, M. Kaufmann, K. J. Lange, R. Reuter, W. Rosenstiel, and A. Zell. 2004. 'ParSeq: searching motifs with structural and biochemical properties', *Bioinformatics*, 20: 1459-61.

Schneewind, O., P. Model, and V. A. Fischetti. 1992. 'Sorting of protein A to the staphylococcal cell wall', *Cell*, 70: 267-81.

Sebulsky, M. T., and D. E. Heinrichs. 2001. 'Identification and characterization of fhuD1 and fhuD2, two genes involved in iron-hydroxamate uptake in Staphylococcus aureus', *J Bacteriol*, 183: 4994-5000.

Shahmirzadi, S. V., M. T. Nguyen, and F. Gotz. 2016. 'Evaluation of Staphylococcus aureus Lipoproteins: Role in Nutritional Acquisition and Pathogenicity', *Front Microbiol*, 7: 1404.

Shang, W., Y. Rao, Y. Zheng, Y. Yang, Q. Hu, Z. Hu, J. Yuan, H. Peng, K. Xiong, L. Tan, S. Li, J. Zhu, M. Li, X. Hu, X. Mao, and X. Rao. 2019. 'beta-Lactam Antibiotics Enhance the Pathogenicity of Methicillin-Resistant Staphylococcus aureus via SarA-Controlled Lipoprotein-Like Cluster Expression', *mBio*, 10.

Sharif, S., M. Singh, S. J. Kim, and J. Schaefer. 2009. 'Staphylococcus aureus peptidoglycan tertiary structure from carbon-13 spin diffusion', *J Am Chem Soc*, 131: 7023-30.

Sheldon, J. R., and D. E. Heinrichs. 2012. 'The iron-regulated staphylococcal lipoproteins', *Front Cell Infect Microbiol*, 2: 41.

Shirtliff, M. E., and J. T. Mader. 2002. 'Acute septic arthritis', *Clin Microbiol Rev*, 15: 527-44.

Silhavy, T. J., D. Kahne, and S. Walker. 2010. 'The bacterial cell envelope', *Cold Spring Harb Perspect Biol*, 2: a000414.

Skaar, E. P., M. Humayun, T. Bae, K. L. DeBord, and O. Schneewind. 2004. 'Iron-source preference of Staphylococcus aureus infections', *Science*, 305: 1626-8.

Skabytska, Y., F. Wolbing, C. Gunther, M. Koberle, S. Kaesler, K. M. Chen, E. Guenova, D. Demircioglu, W. E. Kempf, T. Volz, H. G. Rammensee, M. Schaller, M. Rocken, F. Gotz, and T. Biedermann. 2014. 'Cutaneous innate immune sensing of Toll-like receptor 2-6 ligands suppresses T cell immunity by inducing myeloid-derived suppressor cells', *Immunity*, 41: 762-75.

Smoot, L. M., J. C. Smoot, M. R. Graham, G. A. Somerville, D. E. Sturdevant, C. A. Migliaccio, G. L. Sylva, and J. M. Musser. 2001. 'Global differential gene expression in response to growth temperature alteration in group A Streptococcus', *Proc Natl Acad Sci U S A*, 98: 10416-21.

Sorbara, M. T., and D. J. Philpott. 2011. 'Peptidoglycan: a critical activator of the mammalian immune system during infection and homeostasis', *Immunol Rev*, 243: 40-60.

Spaan, A. N., J. A. G. van Strijp, and V. J. Torres. 2017. 'Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors', *Nat Rev Microbiol*, 15: 435-47.

Stenzel, W., S. Soltek, M. Sanchez-Ruiz, S. Akira, H. Miletic, D. Schluter, and M. Deckert. 2008. 'Both TLR2 and TLR4 are required for the effective immune response in Staphylococcus aureus-induced experimental murine brain abscess', *Am J Pathol*, 172: 132-45.

Stevens, D. L., Y. Ma, D. B. Salmi, E. McIndoo, R. J. Wallace, and A. E. Bryant. 2007. 'Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant Staphylococcus aureus', *J Infect Dis*, 195: 202-11.

Stoll, H., J. Dengjel, C. Nerz, and F. Gotz. 2005. 'Staphylococcus aureus deficient in lipidation of prelipoproteins is attenuated in growth and immune activation', *Infect Immun*, 73: 2411-23.

Sun, Y., A. G. Hise, C. M. Kalsow, and E. Pearlman. 2006. 'Staphylococcus aureus-induced corneal inflammation is dependent on Toll-like receptor 2 and myeloid differentiation factor 88', *Infect Immun*, 74: 5325-32.

Sutcliffe, I. C., and R. R. Russell. 1995. 'Lipoproteins of gram-positive bacteria', *J Bacteriol*, 177: 1123-8.

Suzuki, T., J. Campbell, J. G. Swoboda, S. Walker, and M. S. Gilmore. 2011. 'Role of wall teichoic acids in Staphylococcus aureus endophthalmitis', *Invest Ophthalmol Vis Sci*, 52: 3187-92.

Switalski, L. M., J. M. Patti, W. Butcher, A. G. Gristina, P. Speziale, and M. Hook. 1993. 'A collagen receptor on Staphylococcus aureus strains isolated from patients with septic arthritis mediates adhesion to cartilage', *Mol Microbiol*, 7: 99-107.

Sychantha, D., C. S. Jones, D. J. Little, P. J. Moynihan, H. Robinson, N. F. Galley, D. I. Roper, C. G. Dowson, P. L. Howell, and A. J. Clarke. 2017. 'In vitro characterization of the antivirulence target of Gram-positive pathogens, peptidoglycan O-acetyltransferase A (OatA)', *PLoS Pathog*, 13: e1006667.

Taieb, F., J. P. Nougayrede, and E. Oswald. 2011. 'Cycle inhibiting factors (cifs): cyclomodulins that usurp the ubiquitin-dependent degradation pathway of host cells', *Toxins (Basel)*, 3: 356-68.

Takeda, K., and S. Akira. 2004. 'TLR signaling pathways', *Semin Immunol*, 16: 3-9.

Takeda, K., O. Takeuchi, and S. Akira. 2002. 'Recognition of lipopeptides by Toll-like receptors', *J Endotoxin Res*, 8: 459-63.

Takeuchi, O., K. Hoshino, and S. Akira. 2000. 'Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection', *J Immunol*, 165: 5392-6.

Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda, and S. Akira. 1999. 'Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components', *Immunity*, 11: 443-51.

Takeuchi, O., S. Sato, T. Horiuchi, K. Hoshino, K. Takeda, Z. Dong, R. L. Modlin, and S. Akira. 2002. 'Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins', *J Immunol*, 169: 10-4.

Tarkowski, A. 2006. 'Infection and musculoskeletal conditions: Infectious arthritis', *Best Pract Res Clin Rheumatol*, 20: 1029-44.

Tarkowski, A., L. V. Collins, I. Gjertsson, O. H. Hultgren, I. M. Jonsson, E. Sakiniene, and M. Verdrengh. 2001. 'Model systems: modeling human staphylococcal arthritis and sepsis in the mouse', *Trends Microbiol*, 9: 321-6.

Thomas, M. G., S. Peacock, S. Daenke, and A. R. Berendt. 1999. 'Adhesion of Staphylococcus aureus to collagen is not a major virulence determinant for septic arthritis, osteomyelitis, or endocarditis', *J Infect Dis*, 179: 291-3.

Thompson, B. J., D. A. Widdick, M. G. Hicks, G. Chandra, I. C. Sutcliffe, T. Palmer, and M. I. Hutchings. 2010. 'Investigating lipoprotein biogenesis and function in the model Gram-positive bacterium Streptomyces coelicolor', *Mol Microbiol*, 77: 943-57.

Tjalsma, H., V. P. Kontinen, Z. Pragai, H. Wu, R. Meima, G. Venema, S. Bron, M. Sarvas, and J. M. van Dijl. 1999. 'The role of lipoprotein processing by signal peptidase II in the Gram-positive eubacterium bacillus subtilis. Signal peptidase II is required for the efficient secretion of alpha-amylase, a non-lipoprotein', *J Biol Chem*, 274: 1698-707.

Tokunaga, M., H. Tokunaga, and H. C. Wu. 1982. 'Post-translational modification and processing of Escherichia coli prolipoprotein in vitro', *Proc Natl Acad Sci U S A*, 79: 2255-9.

Tong, S. Y., J. S. Davis, E. Eichenberger, T. L. Holland, and V. G. Fowler, Jr. 2015. 'Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management', *Clin Microbiol Rev*, 28: 603-61.

Torres, V. J., G. Pishchany, M. Humayun, O. Schneewind, and E. P. Skaar. 2006. 'Staphylococcus aureus IsdB is a hemoglobin receptor required for heme iron utilization', *J Bacteriol*, 188: 8421-9.

Travers, J. B., A. Kozman, N. Mousdicas, C. Saha, M. Landis, M. Al-Hassani, W. Yao, Y. Yao, A. M. Hyatt, M. P. Sheehan, A. N. Haggstrom, and M. H. Kaplan. 2010. 'Infected atopic dermatitis lesions contain

pharmacologic amounts of lipoteichoic acid', *J Allergy Clin Immunol*, 125: 146-52 e1-2.

Tristan, A., L. Ying, M. Bes, J. Etienne, F. Vandenesch, and G. Lina. 2003. 'Use of multiplex PCR to identify Staphylococcus aureus adhesins involved in human hematogenous infections', *J Clin Microbiol*, 41: 4465-7.

Tschumi, A., C. Nai, Y. Auchli, P. Hunziker, P. Gehrig, P. Keller, T. Grau, and P. Sander. 2009. 'Identification of apolipoprotein N-acyltransferase (Lnt) in mycobacteria', *J Biol Chem*, 284: 27146-56.

Tsuru, T., and I. Kobayashi. 2008. 'Multiple genome comparison within a bacterial species reveals a unit of evolution spanning two adjacent genes in a tandem paralog cluster', *Mol Biol Evol*, 25: 2457-73.

Walev, I., E. Martin, D. Jonas, M. Mohamadzadeh, W. Muller-Klieser, L. Kunz, and S. Bhakdi. 1993. 'Staphylococcal alpha-toxin kills human keratinocytes by permeabilizing the plasma membrane for monovalent ions', *Infect Immun*, 61: 4972-9.

van Kessel, K. P., J. Bestebroer, and J. A. van Strijp. 2014. 'Neutrophil-Mediated Phagocytosis of Staphylococcus aureus', *Front Immunol*, 5: 467.

Wang, B., and T. W. Muir. 2016. 'Regulation of Virulence in Staphylococcus aureus: Molecular Mechanisms and Remaining Puzzles', *Cell Chem Biol*, 23: 214-24.

Wann, E. R., S. Gurusiddappa, and M. Hook. 2000. 'The fibronectin-binding MSCRAMM FnbpA of Staphylococcus aureus is a bifunctional protein that also binds to fibrinogen', *J Biol Chem*, 275: 13863-71.

Weidenmaier, C., A. Peschel, Y. Q. Xiong, S. A. Kristian, K. Dietz, M. R. Yeaman, and A. S. Bayer. 2005. 'Lack of wall teichoic acids in Staphylococcus aureus leads to reduced interactions with endothelial cells and to attenuated virulence in a rabbit model of endocarditis', *J Infect Dis*, 191: 1771-7.

Venema, R., H. Tjalsma, J. M. van Dijl, A. de Jong, K. Leenhouts, G. Buist, and G. Venema. 2003. 'Active lipoprotein precursors in the Gram-positive eubacterium Lactococcus lactis', *J Biol Chem*, 278: 14739-46.

Verdrengh, M., H. Carlsten, C. Ohlsson, and A. Tarkowski. 2006. 'Rapid systemic bone resorption during the course of Staphylococcus aureus-induced arthritis', *J Infect Dis*, 194: 1597-600.

Verdrengh, M., and A. Tarkowski. 1997. 'Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus', *Infect Immun*, 65: 2517-21.

Wertheim, H. F., D. C. Melles, M. C. Vos, W. van Leeuwen, A. van Belkum, H. A. Verbrugh, and J. L. Nouwen. 2005. 'The role of nasal carriage in Staphylococcus aureus infections', *Lancet Infect Dis*, 5: 751-62.

Weston, B. F., A. Brenot, and M. G. Caparon. 2009. 'The metal homeostasis protein, Lsp, of Streptococcus pyogenes is necessary for acquisition of zinc and virulence', *Infect Immun*, 77: 2840-8.

Wichgers Schreur, P. J., J. M. Rebel, M. A. Smits, J. P. van Putten, and H. E. Smith. 2011. 'Lgt processing is an essential step in Streptococcus suis lipoprotein mediated innate immune activation', *PLoS One*, 6: e22299.

Widdick, D. A., M. G. Hicks, B. J. Thompson, A. Tschumi, G. Chandra, I. C. Sutcliffe, J. K. Brulle, P. Sander, T. Palmer, and M. I. Hutchings. 2011. 'Dissecting the complete lipoprotein biogenesis pathway in Streptomyces scabies', *Mol Microbiol*, 80: 1395-412.

Wiktor, M., D. Weichert, N. Howe, C. Y. Huang, V. Olieric, C. Boland, J. Bailey, L. Vogeley, P. J. Stansfeld, N. Buddelmeijer, M. Wang, and M. Caffrey. 2017. 'Structural insights into the mechanism of the membrane integral N-acyltransferase step in bacterial lipoprotein synthesis', *Nat Commun*, 8: 15952.

Volz, T., M. Nega, J. Buschmann, S. Kaesler, E. Guenova, A. Peschel, M. Rocken, F. Gotz, and T. Biedermann. 2010. 'Natural Staphylococcus aureus-derived peptidoglycan fragments activate NOD2 and act as potent costimulators of the innate immune system exclusively in the presence of TLR signals', *FASEB J*, 24: 4089-102.

Votintseva, A. A., R. Fung, R. R. Miller, K. Knox, H. Godwin, D. H. Wyllie, R. Bowden, D. W. Crook, and A. S. Walker. 2014. 'Prevalence of Staphylococcus aureus protein A (spa) mutants in the community and hospitals in Oxfordshire', *BMC Microbiol*, 14: 63.

Vuong, C., H. L. Saenz, F. Gotz, and M. Otto. 2000. 'Impact of the agr quorum-sensing system on adherence to polystyrene in Staphylococcus aureus', *J Infect Dis*, 182: 1688-93.

Xu, S. X., and J. K. McCormick. 2012. 'Staphylococcal superantigens in colonization and disease', *Front Cell Infect Microbiol*, 2: 52.

Xu, Y., J. M. Rivas, E. L. Brown, X. Liang, and M. Hook. 2004. 'Virulence potential of the staphylococcal adhesin CNA in experimental arthritis is determined by its affinity for collagen', *J Infect Dis*, 189: 2323-33.

Yamagata, H., N. Taguchi, K. Daishima, and S. Mizushima. 1983. 'Genetic characterization of a gene for prolipoprotein signal peptidase in Escherichia coli', *Mol Gen Genet*, 192: 10-4.

Yao, Y., A. Kozman, M. Al-Hassani, C. K. Saha, Q. Yi, W. Yao, N. Mousdicas, M. H. Kaplan, and J. B. Travers. 2010. 'Identification of staphylococcal protein A in infected atopic dermatitis lesions', *J Invest Dermatol*, 130: 2502-4.

Yeowell, H. N., and J. R. White. 1982. 'Iron requirement in the bactericidal mechanism of streptonigrin', *Antimicrob Agents Chemother*, 22: 961-8.

Zahringer, U., B. Lindner, S. Inamura, H. Heine, and C. Alexander. 2008. 'TLR2 - promiscuous or specific? A critical re-evaluation of a receptor expressing apparent broad specificity', *Immunobiology*, 213: 205-24.

Zhu, J., H. Yamane, and W. E. Paul. 2010. 'Differentiation of effector CD4 T cell populations (*)', *Annu Rev Immunol*, 28: 445-89.