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Mini Review

Staphylococcus aureus persistence in non-professional phagocytes

Bettina Löffler*, Lorena Tuchscherr, Silke Niemann, Georg Peters

Institute of Medical Microbiology, University Hospital Münster, Domagkstr. 10, 48149 Münster, Germany

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ABSTRACT

S. aureus is a frequent cause of chronic and therapy-refractory infections. The ability of *S. aureus* to invade different types of non-professional phagocytes, to escape from the host lysosomal degradation machinery and to persist within the intracellular location for long time periods are most likely essential steps in pathogenesis. During the course from acute to chronic infection the bacteria need to dynamically react to the environmental changes and to adapt to the intracellular environment. In this context the bacteria change to SCV-like phenotypes that exhibit some characteristics of stable SCV-mutants, like upregulation of adhesins and downregulation of toxins. The exact formation mechanism and further typical features of these dynamically forming SCVs are largely unknown.

In this review, recent data on the essential steps to establish chronic infections will be summarized and the clinical consequences of the dynamic bacterial adaptation mechanisms will be discussed.

Introduction

Staphylococcus aureus is a major human pathogen that can infect almost every organ and tissue in the body and cause severe and invasive infections (Lowy, 1998). Particularly when these infections enter into a chronic stage, they often become extremely difficult to treat. In many cases even prolonged and combined antimicrobial regimes for several weeks or month fail to clear the infection. Consequently, additional surgery is required, which sets the patients at risk of disability or amputation (Lew and Waldvogel, 2004; Rao et al., 2011; Wright and Nair, 2010). Different types of infections can develop to chronicity; examples that are well known for therapy-failures are osteomyelitis and foreign material-related infections, e.g., vascular graft infections (von Eiff et al., 2006a,b). Whereas in device-associated infections the formation of biofilm on abiotic surfaces is supposed to play a crucial role in protecting the persisting bacteria against the immune system and against antimicrobial treatments (Archer et al., 2011), the pathogenesis of chronic infections in the absence of foreign material appears to be more complex and might originate in the interaction of staphylococci with host tissue (Wright and Nair, 2010).

Traditionally *S. aureus* was considered an extracellular pathogen, but there was increasing evidence that the staphylococci are able to invade various types of host cells. Following host cell invasion, different courses of infection were observed: (i) By releasing toxins and other proinflammatory factors within the intracellular location the staphylococci can induce many inflammatory and cytotoxic effects within their host cells (Bost et al., 1999; Grundmeier et al., 2010; Haslinger-Löffler et al., 2005). (ii) If the intracellular bacteria downregulate or fail to express many secreted virulence factors, then they can persist within morphologically intact host cells for long time periods (Grundmeier et al., 2010; Tuchscherr et al., 2010). The intracellular lifestyle may facilitate long-term persistence in host tissue, as bacteria are largely protected against antimicrobial treatments and the host immune system (Sendi and Proctor, 2009). In this review, the mechanisms and consequences of long-term intracellular persistence will be summarized and their contribution to chronic infections and therapy failures will be discussed.

S. aureus host cell invasion

Tight bacterial adherence to host structures or foreign material is a crucial early step in infection development. Bacteria adhere to host matrix or cells via the expression of adhesive proteins, called adhesins (Clarke and Foster, 2006). *S. aureus* expresses a multitude of adhesins that can be divided into proteins that are covalently bound to bacterial cell wall peptidoglycans (microbial surface components recognizing adhesive matrix molecules, MSCRAMMs) (Foster and Hook, 1998) and proteins that are only secreted but rebind to the bacterial cell surface (secretable expanded repertoire adhesive molecules, SERAMs) (Chavakis et al., 2005). The binding of *S. aureus* to host tissues is a multifactorial process. *S. aureus* uses multiple adhesins for binding to host cells and matrix, which often can mutually compensate the loss-of-function of a given adhesin (redundancy). Nevertheless, the expression of the MSCRAMMs

* Corresponding author at: Institute of Medical Microbiology, University Hospital of Münster, Domagkstraße 10, D-48149 Münster, Germany. Tel.: +49 251 83 55378; fax: +49 251 83 55350.
E-mail address: loeffleb@uni-muenster.de (B. Löffler).

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fibronectin binding proteins (FnBPs) has been described as most important factor for host cell invasion, as disruption of the genes for FnBPs in *S. aureus* largely blocked their ability to be internalized by host cells (Ahmed et al., 2001; Sinha et al., 2000). *S. aureus* FnBPs bind to host cell integrin alpha5beta1 through cellular or soluble fibronectin as a bridging molecule (Sinha et al., 1999; Massey et al., 2001; Fowler et al., 2000). Bacterial adherence to the cellular surface induces changes in the cytoskeleton, which lead to the uptake of the bacteria in phagosomes. This is an active process of the host cells that requires an intact cytoskeleton (Menzies and Kourteva, 1998). In epithelial cells obtained from different anatomic sides the degree of host cell invasion could be partly correlated to the expression of integrin genes (Ridley et al., 2012), suggesting that differences in expression and availability of cellular receptors on the host cell surface may account for the degree of bacterial uptake.

Various cell types have been reported to ingest and degrade *S. aureus*. From the broad literature on *S. aureus* host cell invasion selected work is summarized in Table 1 that mainly addresses invasion, inflammatory effects and long-term persistence. Particularly, epithelial and endothelial cells are known to take up high numbers of bacteria, but also other cell types, such as osteoblasts and fibroblasts, have the capacity to ingest *S. aureus* and shelter the bacteria in their intracellular location (Fig. 1). Even though the skin functions to protect the organisms against invading *S. aureus* strains, keratinocyte can ingest *S. aureus* that can induce cell death (Soong et al., 2011). The bacterial invasion process and the subsequent infection course in different cell types have in common that the uptake mechanism requires an intact cytoskeleton and is an active process of the host cells (Ellington et al., 1999; Kahl et al., 2000; Mempel et al., 2002; Menzies and Kourteva, 1998). Directly after infection bacteria are usually located within phagosomes, but they can later escape to the cytoplasm, which is often associated with cell death induction of host cells (Table 1). Nevertheless, slight differences between cell types (particularly between primary cells and cell lines) regarding host inflammatory processes and bacterial degradation have been reported (Seidl et al., 2012; Tuchscherr et al., 2011) that need to be considered when choosing an infection model.

Early post-invasion inflammatory and cytotoxic effects

Directly after host cell invasion, the bacteria can induce strong inflammatory and cytotoxic effects. These effects are largely dependent on a functioning bacterial accessory gene regulator (Agr)-system (Grundmeier et al., 2010). The Agr-system is a quorum-sensing system that controls the expression of many toxins, exoenzymes and other inflammatory and cytotoxic factors (Kornblum et al., 1990). Particularly, α-hemolysin (α-toxin, Hla) is known as an important toxin that induces inflammation and cell death when expressed within the intracellular location in diverse cell types (Menzies and Kourteva, 2000; Schnaith et al., 2007; Soong et al., 2012) (Table 1). α-Toxin is a pore-forming toxin (Bhakdi and Tranum-Jensen, 1991) that activates and kills mainly mononuclear cells via apoptotic pathways (Bantel et al., 2001; Haslinger et al., 2003), but also acts on epithelial and endothelial cells (Inoshima et al., 2011; Menzies and Kourteva, 2000). Only recently, A-disintegrin and metalloprotease 10 (ADAM10) have been identified as a receptor for α-toxin on epithelial and endothelial cells that mediate loss of barrier functions and tissue injury in acute infection models (Inoshima et al., 2011; Powers et al., 2012). Yet, further binding sides and functions of α-toxin within the intracellular milieu are possible that could be involved in the development of chronic infections.

Additionally, the phenol soluble modulins (PSMs) from *S. aureus* have been described as strong chemotactic and cytolytic peptides for human neutrophils (Wang et al., 2007). PSMs do not only lyse human neutrophils, but also act on different cell types, such as osteoblast, where a killing effect for PSMs has been demonstrated from the intracellular location (Rasigade et al., 2013). Additionally, PSMs have been found to be important virulence factors in disease models (Kretschmer et al., 2010; Chatterjee et al., 2013). Although the human formyl peptide receptor 2 on neutrophils senses PSMs at nanomolar concentrations and initiates an inflammatory response (Kretschmer et al., 2010), PSMs apparently do not require binding to specific proteinaceous receptors for cell lysis. They have high affinity for lipids due to their amphipathic structure, which can compromise the integrity of the plasma membrane (Vandenesch et al., 2012; Wang et al., 2007). Nevertheless, cytolytic activity is only observed for a subset of PSMs and at high concentrations (Wang et al., 2007). Furthermore, these actions of PSMs could be completely blocked by serum lipoproteins (Surewaard et al., 2012), leaving the question open, whether neutrophil cytolysis occurs in vivo or whether the main functions of PSMs is the intracellular lytic activity.

Although the Agr-system largely controls the expression of these important pro-inflammatory and cytotoxic virulence factors (Queck et al., 2008), defects within the Agr-system occur in about 10% of colonizing isolates (Shopsin et al., 2008). Since *agr*$^-$-strains are associated with hospitalization and persistent infections (Park et al., 2013), it is possible that development of *agr*-dysfunction during the infection course might favor chronic and therapy-refractory infections. In vitro testings showed that these strains exhibit a high rate of internalization, but induce less inflammation and cytotoxicity (Grundmeier et al., 2010; Haslinger-Löffler et al., 2005).

Intracellular degradation machineries in non-professional phagocytes and mechanisms of *S. aureus* to escape

Not only professional, but also non-professional phagocytes, such as epithelial or endothelial cells, have the capacity to degrade ingested bacteria. Although these cells do not have the bactericidal endowment as professional phagocytes, they dispose of mechanisms to kill invading pathogens. In particular, autophagy is a catabolic cellular process to remove unwanted or unnecessary material, including pathogens. This most likely represents an important immune mechanism against intracellular bacteria (Deretic, 2005). Pathogens are taken up in autophagosomes and are subjected to the lysosomal machinery (Lerena et al., 2010). Nevertheless, some microorganisms have developed strategies to modulate autophagy to their own benefit and to survive and/or even multiply in autophagic compartments (Campoy and Colombo, 2009). For *S. aureus* it has been demonstrated that some strains expressing *agr*-related factors reveal a marked resistance to autophagic elimination. Thereby, *S. aureus*-containing autophagosomes do not acidify and do not fuse with lysosomes, indicating that *S. aureus* inhibits autophagosome maturation. Further on, *S. aureus* can escape from autophagosomes into the cytoplasm, resulting in host cell death (Schnaith et al., 2007).

Bacterial factors that have been described to mediate the escape from the phagosomes to the cytoplasm are α-toxin and PSMs (Fraunholz and Sinha, 2012; Giese et al., 2011; Schnaith et al., 2007). α-Toxin is a crucial secreted factor that participates in the activation of the autophagic pathway and prevention of autophagosome maturation. Strains deficient in *agr* or *hla*, are unable to activate the autophagic pathway. The phagosomes containing these *agr*$^-$ or *hla*$^-$-bacteria fuse with lysosomes and the microorganisms are eliminated (Mestre and Colombo, 2013). Yet, work by another group in an epithelial cell system revealed that α-toxin is apparently not sufficient to mediate escape from phagolysosomes (Giese et al., 2009). Here, the authors found infected phagolysosomes acidified regardless of α-toxin expression (Giese et al., 2009), whereas intraphagosomal pH levels appeared to be very