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Inside job: *Staphylococcus aureus* host-pathogen interactions

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ABSTRACT

Staphylococcus aureus is a notorious opportunistic pathogen causing a plethora of diseases. Recent research established that once phagocytosed by neutrophils and macrophages, a certain percentage of *S. aureus* is able to survive within these phagocytes which thereby even may contribute to dissemination of the pathogen. *S. aureus* further induces its uptake by otherwise non-phagocytic cells and the ensuing intracellular cytotoxicity is suggested to lead to tissue destruction, whereas bacterial persistence within cells is thought to lead to immune evasion and chronicity of infections. We here review recent work on the *S. aureus* host pathogen interactions with a focus on the intracellular survival of the pathogen.

1. *S. aureus* – a facultative intracellular pathogen?

Staphylococcus aureus is a Gram-positive commensal of humans which persistently colonizes about 10–20% of the human population. Anterior nares and the skin are main sites of *S. aureus* carriage, which often remains asymptomatic. Upon penetration of endothelial, epithelial or dermal barriers, however, the commensal turns into a pathogen. Hence infections with *S. aureus* often are caused by the respective carriage strains (von Eiff et al., 2001). Enabled by a variety of virulence factors as well as an intricate network of regulators by which *S. aureus* adapts to the variety of challenges within different host niches, the pathogen causes a plethora of diseases. These range in their severity from skin abscesses and wound infections to deep tissue abscesses, endocarditis, osteomyelitis, toxic shock syndrome, pneumonia, bacteremia and sepsis (Lowy, 1998).

While originally viewed as exclusively extracellular pathogen, *S. aureus* since has been shown to survive phagocytosis by neutrophils and macrophages and even was shown to be internalized by a variety of otherwise non-phagocytic cells, such as epithelial and endothelial cells, osteoblasts, fibroblasts, etc. (e.g. Strobel et al., 2016). The uptake of the bacteria by non-professional phagocytes is mediated by adhesins. The major adhesins are fibronectin-binding proteins (FnBPs), which indirectly cross link to host receptors such as $\alpha_5\beta_1$ integrins by way of fibronectin bridges (e.g. reviewed in Foster, 2016; Fraunholz and Sinha, 2012). The sequestration of integrins on the host cell surface serves as a signaling stimulus which leads to endocytic uptake of plasma membrane on which *S. aureus* "piggy-backs" into the cells. However, intact epithelia or endothelia usually do not expose pathogen-accessible $\alpha_5\beta_1$ integrins, since these molecules rather are found on the basolateral membrane in the tissues. Only within sub-confluent monolayers of tissue culture cells or upon tissue damage fibronectin receptors are ubiquitously expressed on the cell surfaces in order to facilitate cell contacts and wound closure, respectively. Thus, it may be assumed that intracellular *S. aureus* is found most prevalently close to disrupted epithelia or endothelia in vivo. Tissue destruction further can be caused by cytolytic toxins such as staphylococcal α-toxin. Additionally, α-toxin has been shown to lead to the disruption of barrier function in epithelial cells by interfering with tight junction integrity (Kwak et al., 2012). Further it was shown that E-cadherin cleavage by A Disintegrin and Metalloprotease 10 (ADAM10) was stimulated upon α-hemolysin intoxication of cells (Inoshima et al., 2011). α-toxin also enhanced the expression of β_1 integrin and therefore increased rates of *S. aureus* internalization by and pathogen survival within mast cells (Goldmann et al., 2016). In the latter study, *S. aureus* was shown to upregulate production of fibronectin-binding protein A as well as α-toxin upon exposure to supernatant from degranulated mast cells. By contrast, deletion of the structural gene for α-toxin, *hla*, impaired internalization of the pathogen as did chemical inhibition of ADAM10, the receptor of α-toxin on target cells (Wilke and Bubeck Wardenburg, 2010).

Interestingly, staphylococcal protein A (Spa) also was shown to mediate tissue penetration. In lung epithelia *S. aureus* wild-type but not *spa* mutants were able to spread across polarized airway epithelial cell monolayers via paracellular junctions. Protein A thereby mediated epidermal growth factor receptor (EGFR) and calpain activation as well

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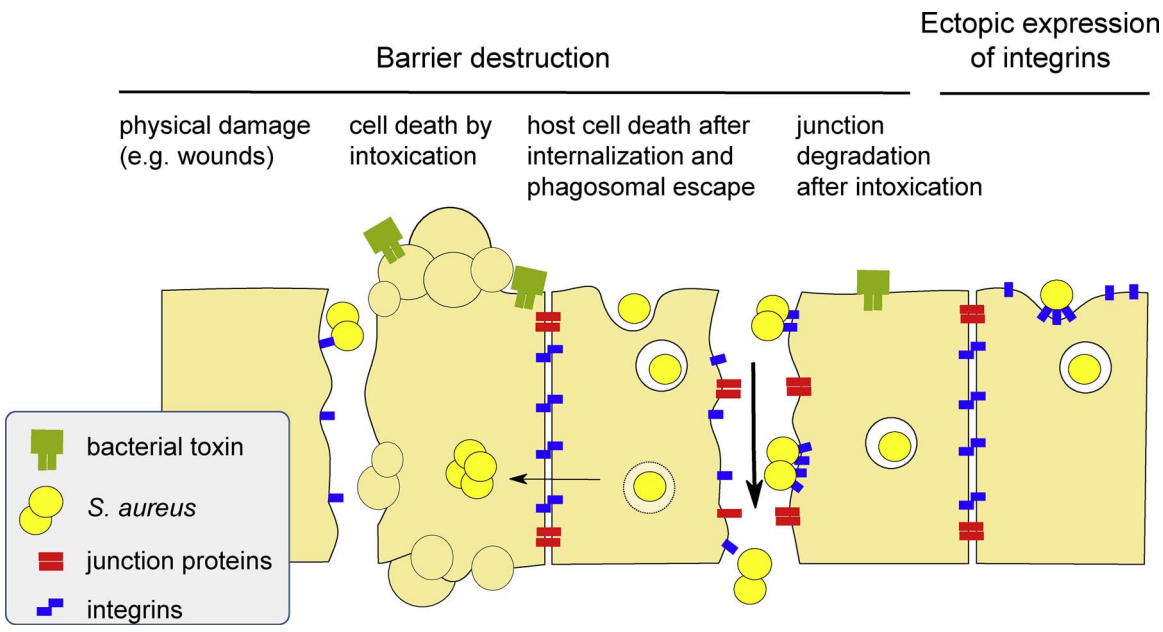

Fig. 1. Possible modes of integrin-dependent internalization of *S. aureus* in vivo. *S. aureus* is efficiently internalized by non-phagocytic cells after binding to integrins. Integrins are exposed in damaged tissue after physical damage, intoxication of cell by bacterial cytolysins, degradation of junction proteins, or after ectopic integrin expression. See text for details.

as cleavage of the junctional proteins occludin and E-cadherin. Thereby it stimulated a RhoA-dependent signaling cascade, which led to cytoskeletal rearrangements (Soong et al., 2011). Thus, *S. aureus* possesses alternative strategies that enable breaching of barriers regardless of the activation state of its main virulence regulator, the quorum-sensing *agr* system, since α-toxin production is upregulated by active *agr,* whereas protein A is produced when *agr* is inactive. Other deleterious effects on barrier functions were attributed to staphylokinase (Sak), which was shown to be involved in skin penetration (Kwiecinski et al., 2014) and hyaluronidase (HysA), which was recognized as a spreading factor in experimental lung infections (Ibberson et al., 2014)

Further, β_1-integrins were recently identified to be ectopically expressed on the apical surface of luminal airway epithelial cells in cystic fibrosis (CF) (Li et al., 2017) thereby possibly explaining the persistent colonization of lungs of CF patients with *S. aureus*. Taken together, it thus is likely that *S. aureus* encounters fibronectin-receptors or alternative adhesins in vivo and may be taken up in ensuing endocytic/phagocytic events in *S. aureus* infections (summarized in Fig. 1).

In vivo evidence for intracellular bacteria comes from a growing number of studies. For example, polymorphonuclear neutrophils (PMN) isolated from the site of infection in murine infection models contain viable intracellular *S. aureus*, which were sufficient to establish infection in naive mice (Gresham et al., 2000). Further, using intravital two-photon microscopy in LysM-eGFP transgenic mice not all *S. aureus* cells are targeted by the green-fluorescent phagocytes. While these bacteria may be quiescent and hence not attract the neutrophils, they also might reside within unstained tissue cells (Liese et al., 2013). Intracellular *S. aureus* also have been detected in human samples such as nasal polyp biopsies (Hayes et al., 2015), in the nasal mucosa of patients with recurrent sinusitis (Clement et al., 2005; Ou et al., 2016a; Plouin-Gaudon et al., 2006), and even within tissue from the nasal vestibule of healthy carriers (Hanssen et al., 2017). The authors of the latter study hypothesized that these intracellular states may protect the bacteria from decolonization procedures, e.g. with the topical agent mupirocin, and hence may account for the quick re-colonization after “eradication” with the original colonizing strain. *S. aureus* further has been shown to survive in CF macrophages thereby forming a reservoir for chronic lung infection in patients, whereas no bacteria were detected in healthy donor controls. The less efficient antibacterial activity in CF macrophages is based on a failure of phagolysosomal fusion due to the absence or incorrect targeting of cystic fibrosis transmembrane conductance regulator (CFTR, Li et al., 2017). Thus, it is likely that intracellular bacteria form an intracellular reservoir of *S. aureus* in humans and thereby contribute to persistence of carriage, chronicity of infection, development of antibiotic resistance and even dissemination from the site of infection to secondary abscesses and so on.

2. Small colony variants and intracellular persistence of *S. aureus*

Once internalized by host cells *S. aureus* can reside within cells for extended periods (Hamill et al., 1986; Kubica et al., 2008; Lowy et al., 1988; Tuchscherr et al., 2011) and bacterial survival within such long-lived host cells is even supposed to support chronicity of *S. aureus* infections. Bacteria that are recovered from sites of long-term infections usually possess reduced metabolic activity accompanied by slow growth rates and increased antibiotic resistance (Proctor et al., 1994; reviewed in Proctor et al., 2006; Sendi and Proctor, 2009). This small colony variant (SCV) phenotype is characterized by distinct changes in transcriptome and proteome of the pathogen (Garzoni et al., 2007; Kriegeskorte et al., 2011). SCV are often non-hemolytic, non-cytotoxic, weakly pigmented and display a thick cell wall (Bulger and Bulger, 1967; Proctor et al., 1994). The reduced hemolysis and cytotoxicity of SCV results from low expression of *agr*. Hence SCV secrete less virulence factors, whereas cell wall-linked adhesins are overproduced (Tuchscherr et al., 2015; Tuchscherr et al., 2011). Especially *S. aureus* isolates from chronic osteomyelitis are therefore characterized by their high host cell invasion rates and adaptation to the intracellular milieu (Kalinka et al., 2014). SCV also largely avoid activation of the host immune system (Tuchscherr et al., 2010; Tuchscherr et al., 2011). Immune responses to SCVs mainly induced TLR2 signaling, whereas wild-type bacteria with normal colony morphology induced the production of cytokines IL-1-β, IL-6 and IL-12 as well as tissue remodeling factors (Ou et al., 2016b). Next to *agr*, the alternative sigma factor σB recently was shown to play a crucial role for mediating the switch from the toxin-producing phenotype to the metabolically quiescent, persisting SCV phenotype. σB mutants were unable to form SCVs and were cleared within days by the host in an infection model rendering σB crucial for adaption to the host environment during chronic infections and for establishing intracellular persistence of *S. aureus* (Tuchscherr et al., 2015). Mutants in *agr* and *sar*A, which are often found at high