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Intracellular Staphylococcus aureus: live-in and let die

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**Keywords:** Staphylococcus aureus, phagocytosis, phagosomal escape, autophagy, host cell death, bacterial persistence.

**INTRODUCTION**
Most manifestations of Staphylococcus aureus disease involve extracellular bacteria (furuncles, carbuncles, impetigo, abscesses, septicemia, necrotizing pneumonia) or biofilm formation (catheter-induced infective endocarditis, atherosclerosis). Aside from this, S. aureus infections have a second face: there is accumulating evidence that S. aureus is able to survive within its host cells and thus might be termed a facultative intracellular pathogen. Intracellularity of S. aureus has been implied as immune-erasive strategy thereby escaping detection by professional phagocytes.

**INTERNALIZATION OF S. aureus BY HOST CELLS**
Invasion of non-professional phagocytes by S. aureus is mediated by a zipper-type mechanism. To date many bacterial adhesives have been identified with Fibronectin (Fn)-binding proteins A and B (FnBPA, FnBPB) constituting the major staphylococcal adhesions for non-professional phagocytes such as epithelial, endothelial cells, fibroblasts, osteoblasts, and keratinocytes (Dziennowska et al., 1999; Ivron et al., 1999; Lammers et al., 1999; Peacock et al., 1999; Sinha et al., 1999; Fowler et al., 2000; Ahmed et al., 2001; Kintarak et al., 2004; Sinha and Fraunholz, 2005, 2008). Clumping factor B (ClfB) has been shown to bind to cytokeratins in the extracellular matrix (ECM) of host cells (O’Brien et al., 2002; Wertheim et al., 2008; Haim et al., 2010), and staphylococcal protein A can directly interact with tumor necrosis factor a receptor 1 (TNFRI; Clar et al., 2011). To what extent the internalization of the pathogen is mediated by WTA, CIB, protein A, and a body of other molecules interacting with the ECM of host cells is not known thus far.

Since FnBPs contribute to the adherence of S. aureus to intact endothelium in vivo (Lachle et al., 2005; Kerdudou et al., 2006; Edwards et al., 2010), we can assume that staphylococcal invasion of epithelia or the endothelium is relevant in natural infections. The interaction of FnBP with ECM Fn is mediated by tandem β zipper structures via the binding of multiple fibronectin molecules by the repetitively arranged modules within a single FnBP (Schwarz-Linek et al., 2003; Rudino-Pineri et al., 2004; Bingham et al., 2008). As a result FnBP/Fn sequester α5β1 integrins on the host cell surface. The resulting receptor clustering relays signals that result in cytoskeletal rearrangements (Agerer et al., 2005; Schröder et al., 2006b). The rearrangements initiated at focal adhesions, which are remodeled to fibrillar adhesions by loss of focal adhesion kinase (FAK), paxillin, and vinculin. The rearrangement is accompanied by a centripetal movement of S. aureus on the host cell surface that were observed by videomicroscopy (Schröder et al., 2006). The repeated generation of actin comet tails beneath adherent staphylococci or FnBα-coated beads and the formation of actin cups without internalization of staphylococci is interpreted by the authors as a delay of phagocytosis (Schröder et al., 2006; Figure 1, Map Item 2). Invasion signaling further involves src kinase (Agerer et al., 2003). Extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK) but not mitogen-activated protein kinase (MAPK) p38 are required in osteoblasts (Ellington et al., 2001), whereas in HEp-2 cells p38 MAPK was found to be upregulated for establishment of nasal colonization and there is evidence that a scavenger receptor is involved in WTA binding (Weidenmaier et al., 2004, 2005, 2008). Clumping factor B (CIB) has been shown to bind to cytokeratins in the extracellular matrix (ECM) of host cells (O’Brien et al., 2002; Wertheim et al., 2008; Haim et al., 2010), and staphylococcal protein A can directly interact with tumor necrosis factor a receptor 1 (TNFRI; Clar et al., 2011). To what extent the internalization of the pathogen is mediated by WTA, CIB, protein A, and a body of other molecules interacting with the ECM of host cells is not known thus far.
A map of intracellular fates of S. aureus. (1) α5β1 integrins are sequestered by FnBP-dependent fibronectin cross-linking at focal adhesions. (2) Centripetal movement and loss of FAK lead to development of fibrillar adhesions, at which phagocytic cups are formed and bacteria are eventually endocytosed. (3) Assembly of α-toxin pores on the plasma membrane of host cells leads to the release of cytochrome c and thus inducing the apoptosome in a Bax-independent pathway of intrinsic apoptosis. Caspase 9 subsequently activates executioner caspases. (8) Cathespin B, a cysteine protease, is released upon bacterial escape from the phagosomal membrane. (9) Caspase-1-mediated pyroptosis occurs in a Bax-independent manner. (10) Phagosomes containing toxic-permeabilized endocytic vesicles are targeted by autophagy. During autophagy, an isolation membrane engulfs leaky endosomes or cytoplasmically located bacteria. Within these autophagosomes, bacteria replicate and eventually escape, ultimately leading to host cell death. ADAM, a metalloprotease and disintegrin; ARP2/3, actin-related protein 2 and 3; ATL, autolysin; CytC, cytochrome c; Eap, extracellular adherence protein; FAK, focal adhesion kinase; FnBP, fibronectin-binding protein; HSP, heat shock protein; IL, interleukin; NFκB, nuclear factor κB; NWASP, neural Wiskott-Aldrich syndrome protein; PAX, paxillin; SR, scavenger receptor; VCL, vinculin; WTA, wall teichoic acid.

Staphylococcus aureus INTRACELLULAR PERSISTENCE AND GROWTH

The fate of the pathogen and the infected host cell depend on staphylococcal isolate and genotype (Cout et al., 2003) as well as differential susceptibility of host cells to virulence factors, host cell gene expression, etc. For example, S. aureus produces different hemolysins. The majority of bovine mastitis strains were phenotypically positive for the sphingomyelinase β-toxin, whereas only a minority of human strains isolated from cases of septicemia...
or nasal carriage was positive for β-toxin (Aarestrup et al., 1999). There seems to exist a selective pressure for S. aureus strains colonizing humans to acquire β-toxin converting phage (Goecke et al., 2009). This is most likely due to staphylococcal complement inhibitor SCIN and the chemotaxis inhibitor protein CHIPS (and additional factors) that are usually found to be encoded by the respective phage genomes. Purified β-toxin, selectively kills monocytes (Bhadki et al., 1996) and destroys platelets, but barely affects other cell types (Wadstrom and Molby, 1972). Similarly, most human cell types are fairly insensitive to the pore-former α-toxin, whereas human leukocytes and cells from other mammalian species are highly susceptible (Bhadki and Tramun-Jensen, 1991). The metalloprotease ADAM10 has been recently identified as receptor of α-toxin monomers (Wille and Buckeb Wardenburg, 2010; Inoshima et al., 2011; Figure 1, Map Item 3). The differential specificities of α-toxin possibly reflect ADAM10 expression differences in the respective cells or might result from different capabilities of host cells to remove α-toxin pores by endocytosis (Husmann et al., 2009).

Staphylococcus aureus survival within host cells was highly dependent on multiplicity of infection (MOI; e.g., Mohammed et al., 2007; Schwartz et al., 2009; Fang et al., 2010) and also the growth phase of the bacteria used for infection (Schwartz et al., 2009). Green fluorescent protein (GFP)-expressing S. aureus displayed bleaching of the fluorescent protein, which indicated degradation of the bacteria in polymorphonuclear neutrophils (PMN; Figure 1, Map Item 4a). The loss of fluorescence was not strain-specific and was seen in each of several different strains of S. aureus, including nosocomial and community-associated methicillin-resistant strains. When rapidly growing S. aureus was used for infection, the bacteria were found to be more susceptible to GFP bleaching (Schwartz et al., 2009). This indicated that these bacteria were cleared more efficiently. Bacterial disinfection was mainly dependent on hypochlorous acid (HOCl; Schwartz et al., 2009; Pang et al., 2010) and also to GFP bleaching (Schwartz et al., 2009). This is further corroborated by the identification of cytoprotective effects on macrophages after phagocytosis of S. aureus. Thus, the up-regulation of anti-apoptotic factors upon staphylococcal infection is responsible for extended phagocyte lifetime (KoezI et al., 2009). Both studies suggest that S. aureus might penetrate deeper into the tissue and even disseminate to different sites within “Trojan horse” phagocytes (KoezI et al., 2009). Survival within PMN is reported to depend on the accessory regulator SarL, which was crucial to S. aureus survival inside spacious vacuoles, whereas sar− strains were localizing to so-called “tight vacuoles” (Gresham et al., 2000). Such different vacuoles can also be observed in non-professional phagocytes (Sinha and Fraunholz, 2010). The large vacuoles also are reminiscent of spacious Listeria-containing phagosomes, which were found to be non-acidified and non-degradative in macrophages (Birmingham et al., 2008). However, a more thorough characterization of the different vacuolar locations in intracellular S. aureus infections is lacking.

Staphylococcus aureus PHAGOSOMAL ESCAPE

Phagocytosed bacterial pathogens evade lysozomal killing, e.g., by disintegration of the organelle membrane in order to translocate into the host cell cytoplasm. Listeria monocytogenes co-opts the pore-forming toxin (PFT) listeriolysin O (LLO) and phospholipases (reviewed in Drami and Cossart, 2002; Schnupf and Dramsi, 2002; Jarry and Cheung, 2006; Kubica et al., 2008). Phagosomal escape of S. aureus initially has been described by Bayles et al. (1998) and the agr-dependency of this process has been demonstrated (Qui et al., 2001; Shompole et al., 2003; Jarry and Cheung, 2006; Kubica et al., 2008). The membrane destructive function of staphylococcal α-toxin thus suggested an involvement of the pore former in phagosomal membrane disruption. The requirement for phagosomal escape and intracellular bacterial survival has been reported in CFT-1, a cytic fibrosis (CF) lung cell line (Jarry and Cheung, 2006) as well as in macrophages (Kubica et al., 2008; Figure 1, Map Item 5a). In CFT-1, S. aureus translocates into the cytoplasm in an α-toxin-dependent manner and the bacteria replicate within the
cytoplasm (Kahl et al., 2000; Jarry et al., 2008). However, in the LCSFN cell line complemented with the wild-type CF transmembrane conductance regulator, CFTR, α-toxin has no effect (Jarry and Cheung, 2006). Further, it has been demonstrated that neither α-toxin overexpressing strains (Lâm et al., 2010) nor laboratory strains inducibly expressing α-toxin (Giese et al., 2009, 2011) are capable of releasing staphylococci into the host cell cytoplasm. By expression of the amphiphilic 26 amino-acid (AA) peptide δ-toxin in the non-cytotoxic laboratory strain S. aureus RN4220 S. aureus was capable of escape in rates similar to that of heterologously expressed LLO (Giese et al., 2011). δ-Toxin is encoded by the agr-effector RNAIII and is translated about 1 h after transcription of RNAIII (Balaban and Novick, 1995). It is capable of lysing bacterial protoplasts, lysosomes, lipid spherules, mitochondria, and erythrocytes in vitro in a temperature-independent manner. Its activity and mode of action is comparable to that of non-ionic detergents (Kreger and Bernheimer, 1971; Kreger et al., 1971; Rahal Jr., 1972; Kapral, 1976; reviewed in Verdon et al., 2009).

δ-Toxin is encoded by RNAIII, the agr effector, and thus might constitute an immediate response to space limitation by phagosomal engulfment (Figure 1, Map Item 5b). However, membrane disruption by δ-toxin depended on the presence of the staphylococcal sphingomyelinase, β-toxin (Giese et al., 2011), which cleaves sphingomyelins (SM) to phosphorylcholine and ceramide moieties. δ-Toxin hardly binds to negatively charged phospholipids, binds strongly to liquid-disordered domains and poorly to cholesterol and sphingomyelin liquid-ordered raft domains (Pokorny et al., 2006). In one model, β-toxin thus may cleave SM to ceramides, which tend to accumulate in membrane microdomains. The hydrophobic nature of ceramide-rich domains thus might constitute regions of δ-toxin assembly, which eventually lead to target membrane permeabilization (Figure 2). S. aureus strain USA300 LAC, however, is escape proficient yet does not encode a functional β-toxin due to homogamy of a β-converting phage (Düey et al., 2006). We thus have to hypothesize alternative factors that can act in phagosomal escape, such as a variety of lipases encoded by the staphylococcal genome or phenol-soluble modulins (PSMs; see below). Alternatively, prophages might be lost, e.g., during exposure to phagosomal reactive oxygen species, and then might contribute to phagosomal escape. A similar activation mechanism is used by Streptococcus pneumoniae, which produces hydrogen peroxide and thereby lyses S. aureus by a "remote control" prophage activation (Selva et al., 2009).

For complete hemolysis of erythrocytes in sheep blood agar plates either a shift to 4°C (hot–cold hemolysis), osmotic stress, or synergistic toxins are necessary. β-Toxin belong to the class of PSMα, which initially had been shown to be present within a hot-phenol extraction of S. epidermidis extracts (Ortie et al., 2004) and only later had been identified in S. aureus by sequence homology (Wang et al., 2007). The major groups of PSMs are divided into two operons referred to as PSMα and PSMβ. The PSMα operon is comprised of four open reading frames (ORFs) with approximately 20 AA. PSMβ encodes two ORFs, which are about 40 AA in length (Wang et al., 2007). The expression of PSMβ has
been demonstrated to result in phagosomal escape in a gain-of-function study (Giese et al., 2011). Just like 5-toxin, PSMα and PSMβ are a-grp dependently expressed. It was recently shown that the staphylococcal agr system is confinement induced and thus comprises not only a quorum sensing system but also a diffusion sensing system active at the single cell level (Carnes et al., 2009). α-Toxin and PSMs are hence produced upon phagosomal confinement and thus also low numbers of endocytosed staphylococci should be able to mount a toxin response in order to avoid lysosomal killing. Whereas gain of function assays demonstrated PSMβ activity in phagosomal escape (Giese et al., 2011), in vivo studies suggest a prominent role of PSMα, with the third ORF of the operon, PSMα2, being the most virulent principle (Wang et al., 2007). Despite these incongruities a common theme emerges: amphiphilic PSMs are involved in phagosomal escape. One possibility to explain the observed differences is the postulation of alternative pathways of phagosomal escape mechanisms for S. aureus.

Molecular patterns of pathogens that reside in the cytoplasm of their hosts are detected by nucleotide-binding and oligomerization domain proteins NOD1 and NOD2, which detect γ-D-glutamyl-diaminopimelic acid and muramyl dipeptide, peptidoglycan components of Gram-negative and Gram-positive bacteria. Peptidoglycan binding leads to a conformation change in NOD proteins, which initiates the recruitment of ubiquitin ligases and kinases and ultimately results in nuclear translocation of NfkB and activator protein 1 and expression of inflammatory genes (reviewed in Strober et al., 2006). NOD2 signaling upon S. aureus infection has been found to induce cytokine production (Kapetanovic et al., 2007) and thus might contribute to induction of inflammation, e.g., in the lung (Gomes and Prince, 2008; Figure 1, Map Item 6). NOD2-deficient mice exhibit a delayed inflammatory response and impaired bacterial clearance after infection with S. aureus (Hruz et al., 2009). α-Toxin facilitates NOD2-dependent recognition of S. aureus muramyl dipeptide (Hruz et al., 2009), possibly by interfering with phagosomal integrity. The observation that S. aureus can translocate into the cytoplasm of host cells and grow without an immediately ensuing cell death (e.g., Kubica et al., 2008) illustrates that phagosomal escape is not identical with cytotoxicity. Thus, the link between cell death (e.g., Kubica et al., 2008) and the alternative stress–response sigma-factor σB (Wesson et al., 1998; Qazi et al., 2001; Shompol et al., 2003; Jarri and Cheung, 2006; Kubica et al., 2008), but mainly seem to be independent of SarA (Haslinger-Löffler et al., 2005). Multiple other studies indicate that S. aureus might kill its other types of host cells from within (Bayles et al., 1998; Menzies and Kourteva, 1998; Wesson et al., 1998; Nuzzo et al., 2000; Tucker et al., 2000; Kruis et al., 2003; Haslinger-Löffler et al., 2005, Chattjee et al., 2008; Larry et al., 2008; Kubica et al., 2008; Läm et al., 2010). The virulence factors required for S. aureus-induced apoptosis in endothelial cells depend on agr and the alternative stress–response sigma-factor σB (Wesson et al., 1998; Qazi et al., 2001; Shompol et al., 2003; Jarri and Cheung, 2006; Kubica et al., 2008), although sarA mutants are sensitive to the action of S. aureus α-toxin, however, comparatively low numbers of S. aureus cells with a combined invasive and strongly hemolytic phenotype readily induce apoptotic cell death in HUVEC (Haslinger-Löffler et al., 2005). This suggests that cell death mechanisms are activated from within their intracellular location. The effect is highly specific, since fixed, non-hemolytic, rifampin-treated or weakly invasive staphylococci are not cytotoxic toward endothelial cells (Haslinger-Löffler et al., 2005). 

Phage-encoded Panton-Valentine leukocidin (PVL) predominately destroys leukocytes, although there also is some species specificity for human and rabbit PMN (Löffler et al., 2010). In PMN, PVL induced a rapid caspase-9/3-dependent cell death in vitro (Genestier et al., 2005). The authors further identified to a mitochondrial localization of the PVL toxin. Isolated mitochondria were permeabilized for pro-apoptotic factors such as cytochrome c (CytC) by PVL which suggested that PVL is able to create pores in the mitochondrial outer membrane and thus triggers a caspase-independent mitochondrial pathway of host cell apoptosis (Genestier et al., 2005; Figure 1, Map Item 8). During the intrinsic pathway of apoptosis release of CytC from injured mitochondria leads to activation of apoptosis-activating factor-1 (AIF-AF-1). Oligomerizing CytC/AIF-AF-1 recruits and subsequently activates pro-caspase 9. Caspase 9 then proteolytically activates effector caspases, which finally cleave their respective substrates resulting in membrane blebbing, and DNA fragmentation (reviewed in Rudel et al., 2010). Similarly, α-toxin has been described to activate caspases via the intrinsic death pathway (Bantel et al., 2001; Haslinger et al., 2003) independently of death receptor signaling (CD95/Fas/APO-1). Bcl-2 overexpressing jurkat cells were protected from α-toxin mediated cell death (Bantel et al.,...
Autophagy sequesters cytoplasmic contents via an isolation mem-
in phagosomal escape (Giese et al., 2011) and thus also might act
in cathepsin release.

Some (Petrilli et al., 2007). The pore-forming protein
γ -toxin has been shown to be involved
in autophagy (reviewed in Dorn et al., 2002; Kirkegaard et al., 2004;
Orvedahl and Levine, 2005; Campoy and Colombo, 2009; Ogawa et al., 2011).

Aside from apoptosis, S. aureus is also able to induce pyrocre-
sis (Figure 1, Map Item 9). There, caspase-1 is activated as part
of an inflammasome, which further consists of NOD-like receptor
protein 3 (NLRP3) and the adapter protein, apoptosis-associated
speck-like protein containing a caspase-associated recruitment
domain (ASC). S. aureus can function as a stimulus for NLRP3
(Munoa-Planillo et al., 2009), however, the molecular identity of the
stimulating signal is not known thus far (Mariathasan et al.,
2006; Ting et al., 2008b; Wright and Nair, 2010). Lysosomal per-
meabilization is one NLRP3-activating principle, wherein release of the lysosomal protease cathepsin B into the cytoplasm con-
tributes to NLRP3 activation (reviewed in Willingham and Ting,
2008; Bergbaken et al., 2009). Thus, it seems likely, that lysosomal
rupture or permeabilization by S. aureus toxins releases cathepsin B
which then activates the inflammasome. Indeed staphylococcal
α, β, and γ-hemolysins have been shown to be important activation
of the NLRP3 inflammasome (Gayen et al., 2009; Munoa-Planillo
et al., 2009; Kebaiter et al., 2012). The pore-forming α- and γ-toxins
of S. aureus permeabilize membranes and thus might be involved
in cathepsin release and subsequent inflammasome activation. α-
Toxin further is known to permeabilize the plasma membrane for
potassium ions. In turn, potassium efflux activates the inflamma-
some (Petrelli et al., 2007). β-Toxin has been shown to be involved
in phagosomal escape (Giese et al., 2011) and thus also might act
in cathepsin release.

Staphylococcus aureus AND THE SUBVERSION OF
AUTOPHAGY

Autophagy sequesters cytoplasmic contents via an isolation mem-
brane. Engraftment of cargo by the so-called phagophore forms
double-membrane-bound autophagic vesicles that eventually fuse
with lysosomes to yield autolysosomes. Autophagy serves for
degradation of organelles or self-digestion during nutrient lim-
iting conditions such as starvation and is generally thought to
constitute a cellular survival mechanism. During bacterial infec-
tions autophagy disposes of leaky vesicles or intracellular bacteria,
however, bacterial pathogens have found multiple ways to subvert
autophagy (reviewed in Durey et al., 2002; Kirkegaard et al., 2004;
Levine, 2003; Campoy and Colombo, 2009; Osvedahl and Levine,
2009; Ogawa et al., 2011).

Staphylococcus aureus was shown to interact with autophago-
somes in a rather unique way (Schnaith et al., 2007) when
compared to autophagy-subvertive strategies of other bacterial
pathogens (Campoy and Colombo, 2009; Ogawa et al., 2011).
S. aureus inhibits fusion of phagosomes with lysosomes. It per-
meabilizes HeLa phagosomes by a mechanism dependent on
Staphylococcus-secreted toxins. The leaky phagosomes are targeted
by autophagy and within autophagosomes S. aureus replicates.
Eventually the bacteria escape from their intracellular confine-
ment into the host cell cytoplasm in an a-gr dependent manner
(Schnaith et al., 2007, Figure 1, Map Item 9). Finally, host cell death
is induced, which is independent of a caspase activation cas-
cade but was blocked by overexpression of anti-autophagic Bcl-2.
Induction of autophagy by rapamycin resulted in an increased
number of recovered colony-forming units, whereas inhibition
with wortmann in reduced the colonies recovered from the intra-
cellular environment. S. aureus-induced autophagy resulted in a
vacuolization of the host cell cytoplasm (“Swiss cheese pheno-
type”). a-Gr deficient S. aureus fail to induce autophagy, which
results in maturation of bacteria-containing phagosomes followed
by lysosomal degradation of the pathogens. α-Toxin is able to per-
meabilize membranes for Ca2+ , an inducer of autophagy (Brady
et al., 2007) and autophagy targets phagosomes perforated by a-
toxin in Chinese hamster ovary cells (Mestre et al., 2010). Whereas
latter observation is in line with the results obtained by Schnaith
et al. (2007) it contrasts findings that α-toxin is not sufficient to
permeabilize HeLa phagosomes (Jarry and Cheung, 2006; Giese
et al., 2009; Lin et al., 2010).

CONCLUSION

With about 200-300 virulence factors, S. aureus is able to exert
a multitude of effects upon its eukaryotic host cells. Although
many details have emerged through ground-breaking and recent
research, only a minority of pathogenicity factors of S. aureus has
been functionally annotated to date. Particularly the assessment
of intracellular staphylococcal virulence is often hampered by the
difficulty to discriminate between toxin effects that result from
extracellular or intracellular bacteria, although inducible toxin
expressing might provide valuable tools for molecular dissection
of host-pathogen interactions.

With our advancing knowledge of cell death mechanisms
ground-breaking experiments will have to be re-evaluated in
order to understand the mechanisms of S. aureus-induced host
cell killing. When comparing experimental research originating
from different labs the influence of a body of factors should be
taken into account that could lead to different infection outcomes.

Staphylococcus aureus strain used for infection, its growth phase
at the time of infection (and hence the bacterial growth medium),
as well as MOI are important, whereas on the host side the cell
type and hence the protein profile will drastically influence infec-
tion outcome by altering host cell susceptibility to bacterial toxins,
response to pathogen-associated molecular patterns, expression of
receptors, caspases, and other host factors.

In order to identify activities of single toxins or virulence factors
gain-of-function studies can be useful, e.g., using the a pathogenic
S. carnosus as toxin delivery vehicle. However, such strategies
will not easily identify pathways during which an orchestrated interplay of multiple virulence factors is required. However, novel high-throughput sequencing technologies of transposon insertions (Gawrisch et al., 2009; van Oijen et al., 2009) open up new possibilities for analysis of genome-wide mutant libraries of clinically relevant strains. Using such novel tools we will be able to address a lot of open questions with regard to intracellular staphylococcal infections: do different adherences result in employment of different uptake mechanisms into non-professional phagocytes as these would not subsequently result in different infection outcomes? What is the nature of tight and spacious vacuoles (Ceresi et al., 2000) that are occupied by, for example, saa1−/− and saa1+a+ S. aureus, respectively? Which alternative pathways for phagosomal escape do exist? Which modes of cell death are activated by a single strain in different host cells types or different strains in a single host cell line? There is still a lot to learn about the versatile facultative intracellular pathogen, Staphylococcus aureus.

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novel high-throughput sequencing technologies of transposon insertions (Gawrisch et al., 2009; van Oijen et al., 2009) open up new possibilities for analysis of genome-wide mutant libraries of clinically relevant strains. Using such novel tools we will be able to address a lot of open questions with regard to intracellular staphylococcal infections: do different adherences result in employment of different uptake mechanisms into non-professional phagocytes as these would not subsequently result in different infection outcomes? What is the nature of tight and spacious vacuoles (Ceresi et al., 2000) that are occupied by, for example, saa1−/− and saa1+a+ S. aureus, respectively? Which alternative pathways for phagosomal escape do exist? Which modes of cell death are activated by a single strain in different host cells types or different strains in a single host cell line? There is still a lot to learn about the versatile facultative intracellular pathogen, Staphylococcus aureus.
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