Intracellular Lifestyle of *Staphylococcus aureus*

Subjects: Infectious Diseases

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Staphylococcus aureus (*S. aureus*) is part of the normal skin and nasal microbiota, with approximately 30% of the healthy adult population colonized mainly in the nasopharyngeal cavity. While colonization is usually asymptomatic, a symptomatic infection can occur if there is a breach in the mucosal barrier or skin. The severity of symptomatic infections ranges from superficial skin and soft tissue infections, to devastating complications, such as necrotizing pneumonia, endocarditis, toxic shock syndrome, and sepsis. In the pre-antibiotic era, *S. aureus* bacteremia mortality rates were astonishingly high, ranging between 75% and 83%. Even though antibiotics have reduced this number significantly, *S. aureus* bloodstream infections still account for over 19,000 deaths annually in the United States. With every new antibiotic that is developed, *S. aureus* resistance has been quickly observed. MRSA strains that are resistant to all penicillin-like β-lactam antibiotics pose a particularly serious threat to the community. Two types of MRSA exist: hospital-acquired (HA-)MRSA and community-acquired (CA-)MRSA. CA-MRSA strains are typically regarded as more virulent and can cause infections in otherwise healthy individuals. This notion is further supported by experimental animal studies, whereas HA-MRSA strains are less virulent than CA-MRSA and cause fewer disseminating diseases. Although in animal models, mice are typically not treated with antibiotics, which is unlike a hospital setting.

Staphylococcus aureus MRSA antimicrobial therapy

1. The Infectious Cycle of *S. aureus* in Non-Professional Phagocytes

Intracellular survival of *S. aureus* has been shown in vitro in almost every cell type, including epithelial cells, endothelial cells, osteoclasts, keratinocytes, and fibroblasts [1][2][3][4][5]. However, the infectious cycle caused by the bacteria depends on the cell type that is infected (**Figure 1**). In general, for the invasion of non-professional phagocytes, *S. aureus* employs a zipper-like mechanism that utilizes fibronectin-binding proteins A and B. These proteins attract fibronectin to the bacterial surface and this bacterial-protein complex is subsequently recognized by integrin α5β1, leading to the internalization of staphylococci in non-professional phagocytes [6]. Several additional staphylococcal surface proteins have been shown to promote internalization in mammalian cells, such as clumping factor A (ClfA), iron-regulated surface determinant B (IsdB), and efflux pump tet38 [6]. *S. aureus* can escape from the endosome into the cytosol of the host cell [7]. The bacteria can replicate inside the cytosol, eventually causing cell lysis and escaping into the extracellular environment (**Figure 1**, left panel). Various bacterial mediators have been suggested to play a role in this escape and survival mechanism. The bacterial two-component system (TCS)

accessory gene regulator (Agr), a quorum-sensing regulatory system, has been found to be a key mediator in the process of the endosomal escape of bacteria [8][9][10]. In HeLa cells, Agr is required for autophagy-mediated cytotoxicity and is essential for bacterial escape into the cytoplasm, intracellular replication, and host cell killing [10]. Importantly, this intracellular *S. aureus* survival mechanism was not observed with an Agr mutant strain. The Agr system is a strict regulator for phenol-soluble modulin (PSM)-α, and -β production [11]. Using an elegant method of *S. aureus* detection in the cytosol, it is showed that PSM-α is crucial for the destruction of the endosomal membrane in epithelial and endothelial cells [12]. An intracellular role of PSMs is supported by the fact that serum lipoproteins can protect against PSM-induced host cell lysis, both in vitro and in vivo [13][14]. Thus, Agr and PSMs play important roles in the intracellular survival of *S. aureus* in non-professional phagocytes. However, evidence for intracellular survival in these cell types has only been shown in vitro and there has rarely been any direct visualization of staphylococci persisting in non-professional phagocytes in animal models of infection or in tissues dissected from infected patients.

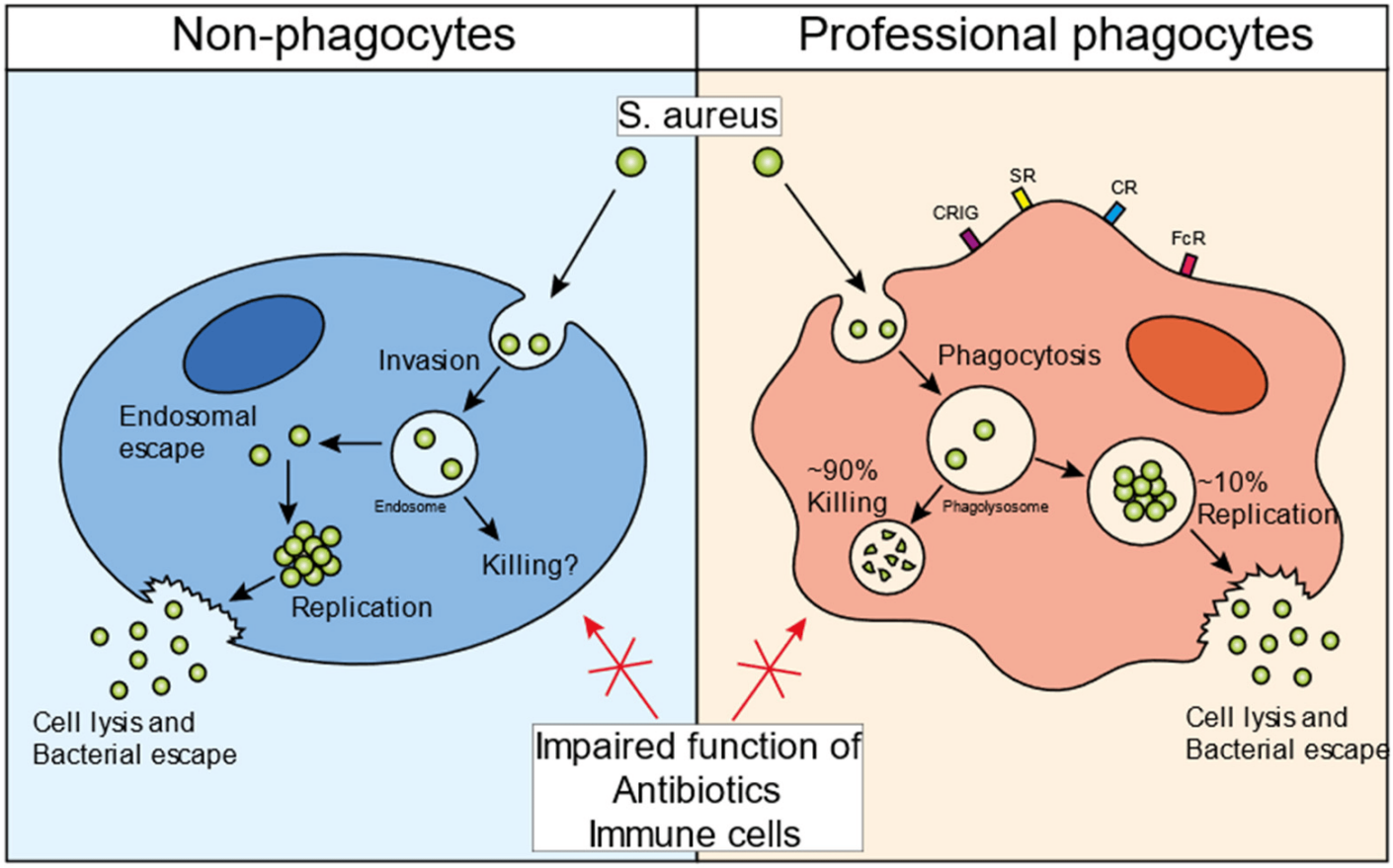

Figure 1. *S. aureus* infectious cycle. Schematic overview of the staphylococcal infectious life cycle in non-professional phagocytes (**left panel**) and professional phagocytes (**right panel**). *S. aureus* invades non-professional phagocytes and ends up inside the endosome. Various bacterial factor regulators, such as Agr and SigmaB, are important for the endosomal escape of the bacteria into the cytosol. Once inside the cytosol, bacteria can persist inside the host cells, maintaining an intracellular bacterial reservoir. Additionally, bacteria can start replicating, eventually lysing the host cell, and escaping into the extracellular environment. Professional phagocytes recognize bacteria with different receptors and take up bacteria by means of phagocytosis (**right**

panel). Once inside the phagolysosome, ~90% of the bacteria are successfully killed. However, the remaining ~10% can evade the intraphagolysosomal killing strategies and then use the cells as a niche to start replicating. Similar to non-professional phagocytes, the bacteria can eventually lyse the host cell and escape into the extracellular environment. The intracellular bacteria in all cell types are protected from immune cells as well as antibiotic treatment. Abbreviations: CRIg, complement receptor of immunoglobulin superfamily; SR, scavenger receptor; CR, complement receptor; FcR, Fc receptor.

2. The Infectious Cycle of *S. aureus* in Professional Phagocytes

Intracellular survival has also been observed in professional phagocytes such as neutrophils and macrophages [15] [16]. There are important differences between these immune cells. Neutrophils are bone marrow-derived, short-lived, and mostly found in the bloodstream, although very limited numbers can be found extravasated in the peripheral tissues. Neutrophils are typically the first cells to arrive at the site of infection and have an incredible capacity for killing bacteria [17]. Macrophages, on the other hand, populate many different tissues and body cavities in large numbers. In homeostasis, macrophages can be derived from yolk-sac or fetal liver progenitors and are self-maintained by local proliferation or by bone marrow-derived monocytes. Upon inflammation, the turnover of macrophages is primarily monocyte-dependent, as monocytes are recruited and differentiate into macrophages. Depending on the tissue they populate, macrophages have very different gene expression profiles and can exert specific cellular functions such as clearance of dead cells, antigen presentation, and coordination of responses to infection [18].

In 1956, a seminal study by David Rogers showed that upon bloodstream infection in rabbits, a small number of bacteria was not removed from the blood by macrophages in the liver, but was internalized by neutrophils [19]. These intracellular bacteria were predicted to be responsible for the large wave of dissemination later in infection. Since then, others have shown that live *S. aureus* can survive in both mouse and human neutrophils and have proposed the “Trojan Horse” theory of dissemination by these immune cells [20]. Intravenous infection of *S. aureus* in mice leads to the uptake of bacteria by neutrophils that carry them in the blood for hours [21]. Isolated neutrophils from an infected animal were sufficient to cause full-blown disease in healthy animals [15]. Furthermore, patients with normal neutrophil counts may be more susceptible to bacterial dissemination compared to patients with reduced neutrophil counts [22]. Although neutrophils have been demonstrated to enable the persistence of *S. aureus* during infection, eventually leading to bacterial escape, there is limited experimental in vivo evidence that replication occurs inside these cells [15].

In contrast to neutrophils, there is an increasing body of in vivo evidence that intracellular replication occurs in macrophages. During bloodstream infection, the vast majority of staphylococci are taken up in the liver by the largest population of resident macrophages in the body, called Kupffer cells (KCs). KCs are a self-sustaining population of macrophages strategically situated within the liver sinusoids and in direct contact with the blood circulation [23]. In contrast to many other tissue macrophages and neutrophils, KCs are able to catch bacteria directly out of the bloodstream and overcome the high shear forces of blood flow [24]. KCs are crucial cells during

bloodstream *S. aureus* infections, and KC depletion leads to massive, sustained bacteremia, and a greatly increased mortality due to *S. aureus* infections. Since KCs can kill ~90% of the injected inoculum, they are regarded as an important initial immune bottleneck during staphylococcal bloodstream infections, whereas neutrophils are essential at later time points [25]. Various studies have shown that not all staphylococci succumb to the intracellular bactericidal phagolysosome of KCs, and high-resolution intravital microscopy in combination with replication-reporter bacteria showed that staphylococci can replicate within these cells over time [26][27][28][29][30].

Using intravital microscopy in living animals, it was shown that *S. aureus* replicates in approximately 10% of infected KCs. Microcolonies of approximately 50–70 bacteria can be observed to lead to the destruction of the KC, which is followed by dissemination to various other internal organs, primarily the kidney (**Figure 1**, right panel). Mechanistically, *S. aureus* was found to replicate in phagolysosomes from KCs that did not produce sufficient reactive oxygen species (ROS) to kill the intracellular bacteria [26]. Interestingly, these replicating staphylococci induced α-toxin expression, which is released back into the circulation, thereby causing platelet aggregation, microthrombus formation, vascular occlusion, and organ dysfunction [31]. Even though α-toxin was upregulated by *S. aureus* in KCs, it remains to be determined which staphylococcal toxin is responsible for the lysis of Kupffer cells. Jorch et al. showed that after *S. aureus* escapes from the KCs, they relocate from the liver into the peritoneal cavity, where they get phagocytosed by Gata6^{+} peritoneal macrophages [32]. Similar to KC infections, *S. aureus* can persist inside the peritoneal macrophages and disseminate to organs in the peritoneal cavity, including the kidneys. Collectively, these data show that in the murine bloodstream infection model, *S. aureus* can infect and escape from multiple macrophage subtypes, ultimately seeding into the kidney, where it establishes classical abscesses 3–4 days post-infection. In a murine airway infection model, macrophages were also essential for the clearance of *S. aureus* since the loss of alveolar macrophages inhibited the killing of bacteria and significantly enhanced mortality [33]. Some studies have shown that *S. aureus* is actively taken up by alveolar macrophages, although it is currently unclear whether *S. aureus* replication occurs in this population of resident macrophages.

3. Intraphagolysosomal Evasion Strategies of *S. aureus*

Unlike bacterial invasion observed upon the infection of non-professional phagocytes, professional phagocytes engage in a variety of phagocytic receptors to actively engulf and internalize bacteria via phagocytosis (**Figure 1**, right panel). During phagocytosis, the microbe is enclosed in a bacteria-containing phagosome within the phagocytic cell. These phagosomes undergo several maturation steps to ultimately become phagolysosomes, a process in which the interior of the vesicle progressively increases its acidity. Phagolysosomes contain a variety of antimicrobial peptides and degradative lysosomal enzymes, and are bombarded with ROS, all of which contribute to the killing of ingested bacteria [34]. The microbicidal activity of the phagolysosome is incredibly disruptive to most bacteria, and in order to establish an infection, many pathogens have evolved various strategies to neutralize or resist the effector components of the phagolysosome. Nonetheless, for *S. aureus*, there is some controversy on how these bacteria can overcome the phagosomal effector functions. Some literature suggests that the phagosomal maturation pathway is hijacked by *S. aureus* [30][35], while most papers found normal phagosomal maturation suggesting that *S. aureus* can withstand the harmful environment of the phagolysosome [12][26][29][36][37]

[38][39]. *S. aureus*, therefore, must overcome these defensive mechanisms in order to enable intracellular replication. Unsurprisingly, the bacteria express a plethora of evasion molecules inside the phagolysosome (**Figure 2**).

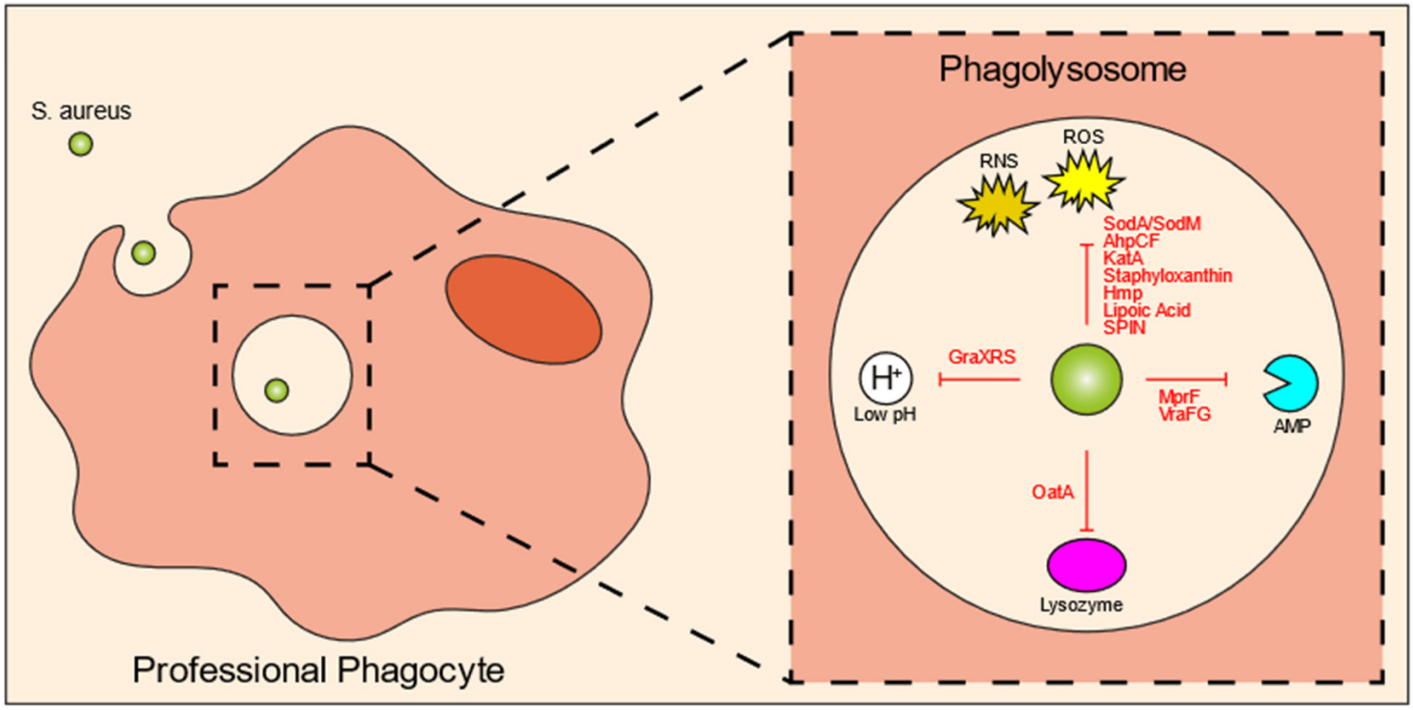

Figure 2. *S. aureus* intraphagolysosomal evasion strategies. Once inside the phagolysosome of professional phagocytes, the bacteria get exposed to various antimicrobial molecules designed to kill the bacteria. Co-evolution of host and pathogen has led to a variety of evasion molecules produced by *S. aureus* to counteract these phagolysosomal killing mechanisms. These mechanisms include evasion molecules against ROS and RNS, AMPs, lysozyme, and acidic pH. Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; Sod, superoxide dismutase; AhpCF, alkyl hydroperoxidase reductase; KatA, catalase; Hmp, flavohemoglobin; SPIN, staphylococcal peroxidase inhibitor; AMP, antimicrobial peptide; MprF, multiple peptide resistance factor; OatA, O-acetyltransferase.

One of the most important killing mechanisms of the host cells is ROS, which is produced by NADPH oxidase (NOX2) via catalyzation of superoxide (O_2-). Oxygen radicals have great antimicrobial activity, targeting various bacterial components, such as DNA, proteins, and the bacterial cell membrane. Nitrogen radicals can also be created by immune cells, termed reactive nitrogen species (RNS). It is not surprising that most *S. aureus* evasion molecules are directed against these molecules (**Figure 2**). SodA and SodM are staphylococcal superoxide dismutases that directly eliminate ROS [40][41]. Similarly, alkyl hydroperoxidase reductase (AhpCF) and catalase (KatA) directly incapacitate peroxides and H_2O_2, respectively [42][43]. Staphyloxanthin is a carotenoid pigment with strong antioxidant properties [44]. For nitric oxide resistance, *S. aureus* can produce flavohemoglobin (Hmp), which acts like a denitrosylase [45]. ROS and RNS production can be inhibited by lipoic acid [46], and staphylococcal peroxidase inhibitor (SPIN) can bind to and block myeloperoxidase (MPO), which is the enzyme that converts H_2O_2 and chloride into hypochlorous acid [47].

A recent study by Leliefeld et al. using an in vitro fibrinogen gel model to increase the lifespan of neutrophils, showed that neutrophils limit staphylococcal growth in a pH-dependent manner [48]. Contrarily, in macrophages, it was shown that the intraphagolysosomal acidic pH is important for *S. aureus* to enable replication. This was orchestrated by GraXRS, which has been shown both in vitro as well as in vivo [49][50]. Similar to survival inside non-professional phagocytes, SigmaB could play a role in intraphagosomal survival, because a wild-type *S. aureus* strain showed increased resistance to acidic pH and hydrogen peroxide, when compared to a SigmaB-inactivated mutant in vitro [51]. In line with this observation, the SigmaB activating protein RsbU has been shown to be necessary for the intracellular growth of *S. aureus* within the phagolysosomes of THP-1 macrophages and HUVEC cells [52]. Various host cell-derived enzymes are released into the phagolysosome, among which are proteases. Lysozyme, which can cleave bacterial peptidoglycan and subsequently induce bacterial cell lysis, is particularly harmful. In response, *S. aureus* produces O-acetyltransferase (OatA) that acetylates peptidoglycan so it can no longer be targeted by lysozyme [53][54]. Finally, there are other antimicrobial peptides released into the phagolysosomal environment, many of which are positively charged. Multiple peptide resistance factors (MprF) of *S. aureus* catalyze a reaction that attaches a positively-charged lysine to the negatively-charged lipids of the bacterial membrane, thereby reducing interaction with antimicrobial peptides, defensins and protegrins in particular [55][56][57]. VraFG is a transporter of antimicrobial peptides and helps with resistance against cationic antimicrobial peptides [58].

Not all studies suggest that *S. aureus* replicates within the phagolysosome. Kubica et al. reported that *S. aureus* escaped into the cytosol of macrophages using α-toxin, where they replicated for a few days followed by host cell lysis. Several *S. aureus* factors were seen to be important for this survival, including SigmaB and Agr [59]. Another study suggested that the *S. aureus* USA300 strain can disturb normal phagolysosome formation and can sense the intraphagolysosomal pH in order to escape into the cytosol and commence intracellular replication [29]. Staphylococcus-produced PSMs have been shown to be important for killing neutrophils after phagocytosis. These toxins are actively upregulated by the bacteria within the phagolysosome by Agr and the stringent response system, and both systems appear to be crucial for the intracellular production of PSMs and escape from neutrophils [60][61].

4. Bacterial Specificity of the Intracellular Reservoir

Collectively, research has shown that *S. aureus* can persist and replicate in multiple cell types, maintaining the bacterial population during infection, and leading to the dissemination of bacteria throughout the body. Commensal bacteria are typically cleared by host phagocytes after infection, whereas non-aureus staphylococci can persist in macrophages but do not replicate [62]. Co-infection of *S. aureus* and commensal bacteria only led to the proliferation of *S. aureus* within KCs [63]. The commensal bacteria augment *S. aureus* infection by acting as a sink for ROS production, thereby increasing the chance that *S. aureus* can initiate replication inside KCs [64]. Many other bacterial, fungal, or parasitic species have been shown to use circulating phagocytes as an opportunity for dissemination, such as *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Leishmania*, and *Cryptococcus neoformans* [65][66][67][68].

The ability to persist and replicate intracellularly might be *S. aureus* strain specific. For example, where the Newman strain has been shown to be acid sensitive and is cleared in an intracellular infection [69], the more virulent USA300 strain can survive in phagocytes and has even been suggested to require acidification of the phagolysosome for persistence [50]. In a study where different *S. aureus* strains (Cowan I, 6850, Novel, Wood 46, BS 507, BS 513, BS 800, BS 890, BS 891, and RN4220) were tested in vitro with different cell lines (HEK-293, HeLa, and EA.hy926 cells), it was observed that all strains survived inside fully acidified vesicles except for *S. aureus* 6850, which was present in vesicles with reduced acidification [39]. Xiong et al. compared genotypes of persistent *S. aureus* strains with strains associated with rapid clearance in the blood and showed that persistence was associated with overexpression of Agr, staphylococcal cassette chromosome mec type II, clonal complex 30, and spa 16 [70]. Hence, only some *S. aureus* strains have the unique ability to persist and grow inside host phagocytes. As outlined above, various virulence mechanisms have implicated the ability of *S. aureus* to withstand the innate defenses of neutrophils and macrophages, even though not all studies are in agreement. The discrepancy between studies could be due to staphylococcal strain variation, use of distinct cells and or cell lines, and methodological differences. It should also be noted that in vitro generated data might be far from the actual in vivo survival mechanisms of *S. aureus* within macrophages. Nevertheless, it is safe to assume that there is a redundancy in evasion strategies that provides the bacteria time to adapt to the intraphagolysosomal environment to facilitate the intracellular reservoir.

References

1. Bayles, K.W.; Wesson, C.A.; Liou, L.E.; Fox, L.K.; Bohach, G.A.; Trumble, W.R. Intracellular Staphylococcus aureus Escapes the Endosome and Induces Apoptosis in Epithelial Cells. Infect. Immun. 1998, 66, 336–342.
2. Hess, D.J.; Henry-Stanley, M.J.; Erickson, E.A.; Wells, C.L. Intracellular Survival of Staphylococcus aureus within Cultured Enterocytes. J. Surg. Res. 2003, 114, 42–49.
3. Menzies, B.E.; Kourteva, I. Staphylococcus aureus α-Toxin Induces Apoptosis in Endothelial Cells. FEMS Immunol. Med. Microbiol. 2000, 29, 39–45.
4. Nair, S.P.; Bischoff, M.; Senn, M.M.; Berger-Bächi, B. The ΣB Regulon Influences Internalization of Staphylococcus aureus by Osteoblasts. Infect. Immun. 2003, 71, 4167–4170.
5. Rogers, R.; Tompsett, R. The Survival of Staphylococci within Human Leukocytes. J. Exp. Med. 1952, 28, 470.
6. Foster, T.J.; Geoghegan, J.A.; Ganesh, V.K.; Höök, M. Adhesion, Invasion and Evasion: The Many Functions of the Surface Proteins of Staphylococcus aureus. Nat. Rev. Microbiol. 2014, 12, 49–62.
7. Moldovan, A.; Fraunholz, M.J. In or out: Phagosomal Escape of Staphylococcus aureus. Cell. Microbiol. 2019, 21, e12997.

8. Qazi, S.N.A.; Harrison, S.E.; Self, T.; Williams, P.; Hill, P.J. Real-Time Monitoring of Intracellular Staphylococcus aureus Replication. J. Bacteriol. 2004, 186, 1065–1077.
9. Shompole, S.; Henon, K.T.; Liou, L.E.; Dziewanowska, K.; Bohach, G.A.; Bayles, K.W. Biphasic Intracellular Expression of Staphylococcus aureus Virulence Factors and Evidence for Agr-Mediated Diffusion Sensing. Mol. Microbiol. 2003, 49, 919–927.
10. Schnaith, A.; Kashkar, H.; Leggio, S.A.; Addicks, K.; Krönke, M.; Krut, O. Staphylococcus aureus Subvert Autophagy for Induction of Caspase-Independent Host Cell Death. J. Biol. Chem. 2007, 282, 2695–2706.
11. Queck, S.Y.; Jameson-Lee, M.; Villaruz, A.E.; Bach, T.H.L.; Khan, B.A.; Sturdevant, D.E.; Ricklefs, S.M.; Li, M.; Otto, M. RNAIII-Independent Target Gene Control by the Agr Quorum-Sensing System: Insight into the Evolution of Virulence Regulation in Staphylococcus aureus. Mol. Cell 2008, 32, 150–158.
12. Grosz, M.; Kolter, J.; Paprotka, K.; Winkler, A.C.; Schäfer, D.; Chatterjee, S.S.; Geiger, T.; Wolz, C.; Ohlsen, K.; Otto, M.; et al. Cytoplasmic Replication of Staphylococcus aureus upon Phagosomal Escape Triggered by Phenol-Soluble Modulin α. Cell. Microbiol. 2014, 16, 451–465.
13. Surewaard, B.G.J.; Nijland, R.; Spaan, A.N.; Kruijtzer, J.A.W.; de Haas, C.J.C.; van Strijp, J.A.G. Inactivation of Staphylococcal Phenol Soluble Modulins by Serum Lipoprotein Particles. PLoS Pathog. 2012, 8, e1002606.
14. Hommes, J.W.; Kratofil, R.M.; Wahlen, S.; de Haas, C.J.C.; Hildebrand, R.B.; Hovingh, G.K.; Otto, M.; van Eck, M.; Hoekstra, M.; Korporaal, S.J.A.; et al. High Density Lipoproteins Mediate in Vivo Protection against Staphylococcal Phenol-Soluble Modulins. Sci. Rep. 2021, 11, 15357.
15. Gresham, H.D.; Lowrance, J.H.; Caver, T.E.; Wilson, B.S.; Cheung, A.L.; Lindberg, F.P. Survival of Staphylococcus aureus Inside Neutrophils Contributes to Infection. J. Immunol. 2000, 164, 3713–3722.
16. Koziel, J.; Maciag-Gudowska, A.; Mikolajczyk, T.; Bzowska, M.; Sturdevant, D.E.; Whitney, A.R.; Shaw, L.N.; DeLeo, F.R.; Potempa, J. Phagocytosis of Staphylococcus aureus by Macrophages Exerts Cytoprotective Effects Manifested by the Upregulation of Antiapoptotic Factors. PLoS ONE 2009, 4, e5210.
17. Spaan, A.N.; Surewaard, B.G.J.; Nijland, R.; van Strijp, J.A.G. Neutrophils Versus Staphylococcus aureus: A Biological Tug of War. Annu. Rev. Microbiol. 2013, 67, 629–650.
18. Varol, C.; Mildner, A.; Jung, S. Macrophages: Development and Tissue Specialization. Annu. Rev. Immunol. 2015, 33, 643–675; 0324141122.
19. Rogers, D.E. Studies on Bacteriemia I. Mechanisms Relating to the Persistence of Bacteriemia in Rabbits Following the Intravenous Injection of Staphylococci. J. Exp. Med. 1956, 103, 713–742.

20. Thwaites, G.E.; Gant, V. Are Bloodstream Leukocytes Trojan Horses for the Metastasis of Staphylococcus aureus? Nat. Rev. Microbiol. 2011, 9, 215–222.
21. Krezalek, M.A.; Hyoju, S.; Zaborin, A.; Okafor, E.; Chandrasekar, L.; Bindokas, V.; Guyton, K.; Montgomery, C.P.; Daum, R.S.; Zaborina, O.; et al. Can Methicillin-Resistant Staphylococcus aureus Silently Travel from the Gut to the Wound and Cause Postoperative Infection? Modeling the “Trojan Horse Hypothesis”. Ann. Surg. 2018, 267, 749–758.
22. Venditti, M.; Falcone, M.; Micozzi, A.; Carfagna, P.; Taglietti, F.; Serra, P.F.; Martino, P. Staphylococcus aureus Bacteremia in Patients with Hematologic Malignancies: A Retrospective Case-Control Study. Haematologica 2003, 88, 923–930.
23. Krenkel, O.; Tacke, F. Liver Macrophages in Tissue Homeostasis and Disease. Nat. Rev. Immunol. 2017, 17, 306–321.
24. Jenne, C.N.; Kubes, P. Immune Surveillance by the Liver. Nat. Immunol. 2013, 14, 996–1006.
25. Pollitt, E.J.G.; Szkuta, P.T.; Burns, N.; Foster, S.J. Staphylococcus aureus Infection Dynamics. PLoS Pathog. 2018, 14, e1007112.
26. Surewaard, B.G.J.; Deniset, J.F.; Zemp, F.J.; Amrein, M.; Otto, M.; Conly, J.; Omri, A.; Yates, R.M.; Kubes, P. Identification and Treatment of the Staphylococcus aureus Reservoir in Vivo. J. Exp. Med. 2016, 213, 1141–1151.
27. Flannagan, R.S.; Heit, B.; Heinrichs, D.E. Antimicrobial Mechanisms of Macrophages and the Immune Evasion Strategies of Staphylococcus aureus. Pathogens 2015, 4, 826–868.
28. Lacoma, A.; Cano, V.; Moranta, D.; Regueiro, V.; Domínguez-Villanueva, D.; Laabei, M.; González-Nicolau, M.; Ausina, V.; Prat, C.; Bengoechea, J.A. Investigating Intracellular Persistence of Staphylococcus aureus within a Murine Alveolar Macrophage Cell Line. Virulence 2017, 8, 1761–1775.
29. Tranchemontagne, Z.R.; Camire, R.B.; O’Donnell, V.J.; Baugh, J.; Burkholder, K.M. Staphylococcus aureus Strain USA300 Perturbs Acquisition of Lysosomal Enzymes and Requires Phagosomal Acidification for Survival inside Macrophages. Infect. Immun. 2015, 84, 241–253.
30. Jubrail, J.; Morris, P.; Bewley, M.A.; Stoneham, S.; Johnston, S.A.; Foster, S.J.; Peden, A.A.; Read, R.C.; Marriott, H.M.; Dockrell, D.H. Inability to Sustain Intraphagolysosomal Killing of Staphylococcus aureus Predisposes to Bacterial Persistence in Macrophages. Cell. Microbiol. 2016, 18, 80–96.
31. Surewaard, B.G.J.; Thanabalasuriar, A.; Zeng, Z.; Tkaczyk, C.; Cohen, T.S.; Bardoel, B.W.; Jorch, S.K.; Deppermann, C.; Bubeck Wardenburg, J.; Davis, R.P.; et al. α-Toxin Induces Platelet Aggregation and Liver Injury during Staphylococcus aureus Sepsis. Cell Host Microbe 2018, 24, 271–284.

32. Jorch, S.K.; Surewaard, B.G.J.; Hossain, M.; Peiseler, M.; Deppermann, C.; Deng, J.; Bogoslowski, A.; van der Wal, F.; Omri, A.; Hickey, M.J.; et al. Peritoneal GATA6+ Macrophages Function as a Portal for Staphylococcus aureus Dissemination. J. Clin. Investig. 2019, 129, 4643–4656.
33. Kitur, K.; Parker, D.; Nieto, P.; Ahn, D.S.; Cohen, T.S.; Chung, S.; Wachtel, S.; Bueno, S.; Prince, A. Toxin-Induced Necroptosis Is a Major Mechanism of Staphylococcus aureus Lung Damage. PLoS Pathog. 2015, 11, e1004820.
34. Flannagan, R.S.; Cosío, G.; Grinstein, S. Antimicrobial Mechanisms of Phagocytes and Bacterial Evasion Strategies. Nat. Rev. Microbiol. 2009, 7, 355–366.
35. Kahl, B.C.; Goulian, M.; Van Wamel, W.; Herrmann, M.; Simon, S.M.; Kaplan, G.; Peters, G.; Cheung, A.L. Staphylococcus aureus RN6390 Replicates and Induces Apoptosis in a Pulmonary Epithelial Cell Line. Infect. Immun. 2000, 68, 5385–5392.
36. Lowy, F.D. Staphylococcus aureus Infections. N. Engl. J. Med. 1998, 339, 520–532.
37. Flannagan, R.S.; Heit, B.; Heinrichs, D.E. Intracellular Replication of Staphylococcus aureus in Mature Phagolysosomes in Macrophages Precedes Host Cell Death, and Bacterial Escape and Dissemination. Cell. Microbiol. 2016, 18, 514–535.
38. Jarry, T.M.; Cheung, A.L. Staphylococcus aureus Escapes More Efficiently from the Phagosome of a Cystic Fibrosis Bronchial Epithelial Cell Line than from Its Normal Counterpart. Infect. Immun. 2006, 74, 2568–2577.
39. Lâm, T.T.; Giese, B.; Chikkaballi, D.; Kühn, A.; Wolber, W.; Pané-Farré, J.; Schäfer, D.; Engelmann, S.; Fraunholz, M.; Sinha, B. Phagolysosomal Integrity Is Generally Maintained after Staphylococcus aureus Invasion of Nonprofessional Phagocytes but Is Modulated by Strain 6850. Infect. Immun. 2010, 78, 3392–3393.
40. Karavolos, M.H.; Horsburgh, M.; Ingham, E.; Foster, S.J. Role and Regulation of the Superoxide Dismutases of Staphylococcus aureus. Microbiology 2003, 149, 2749–2758.
41. Das, D.; Saha, S.S.; Bishayi, B. Intracellular Survival of Staphylococcus aureus: Correlating Production of Catalase and Superoxide Dismutase with Levels of Inflammatory Cytokines. Inflamm. Res. 2008, 57, 340–349.
42. Cosgrove, K.; Coutts, G.; Jonsson, I.M.; Tarkowski, A.; Kokai-Kun, J.F.; Mond, J.J.; Foster, S.J. Catalase (KatA) and Alkyl Hydroperoxide Reductase (AhpC) Have Compensatory Roles in Peroxide Stress Resistance and Are Required for Survival, Persistence, and Nasal Colonization in Staphylococcus aureus. J. Bacteriol. 2007, 189, 1025–1035.
43. Mashruwala, A.A.; Boyd, J.M. The Staphylococcus aureus SrrAB Regulatory System Modulates Hydrogen Peroxide Resistance Factors, Which Imparts Protection to Aconitase during Aerobic Growth. PLoS ONE 2017, 12, e0170283.

44. Pandey, S.; Sahukhal, G.S.; Elasri, M.O. The MsaABCR Operon Regulates the Response to Oxidative Stress in Staphylococcus aureus. J. Bacteriol. 2019, 201, e00417-19.
45. Nobre, L.S.; Gonçalves, V.L.; Saraiva, L.M. Flavohemoglobin of Staphylococcus aureus. Methods Enzymol. 2008, 436, 203–216.
46. Grayczyk, J.P.; Alonzo, F., III. Staphylococcus aureus Lipoic Acid Synthesis Limits Macrophage Reactive Oxygen and Nitrogen Species Production to Promote Survival during Infection. Infect. Immun. 2019, 87, e00344-19.
47. De Jong, N.W.M.; Ramyar, K.X.; Guerra, F.E.; Nijland, R.; Fevre, C.; Voyich, J.M.; McCarthy, A.J.; Garcia, B.L.; Van Kessel, K.P.M.; Van Strijp, J.A.G.; et al. Immune Evasion by a Staphylococcal Inhibitor of Myeloperoxidase. Proc. Natl. Acad. Sci. USA 2017, 114, 9439–9444.
48. Leliefeld, P.H.C.; Pillay, J.; Vrisekoop, N.; Heeres, M.; Tak, T.; Kox, M.; Rooijakkers, S.H.M.; Kuijpers, T.W.; Pickkers, P.; Leenen, L.P.H.; et al. Differential Antibacterial Control by Neutrophil Subsets. Blood Adv. 2018, 2, 1344–1354.
49. Villanueva, M.; García, B.; Valle, J.; Rapún, B.; Ruiz De Los Mozos, I.; Solano, C.; Martí, M.; Penadés, J.R.; Toledo-Arana, A.; Lasa, I. Sensory Deprivation in Staphylococcus aureus. Nat. Commun. 2018, 9, 523.
50. Flannagan, R.S.; Kuiack, R.C.; McGavin, M.J.; Heinrichs, D.E. Staphylococcus aureus Uses the GraXRS Regulatory System To Sense and Adapt to the Acidified Phagolysosome in Macrophages. MBio 2018, 9, e01143-18.
51. Chan, P.F.; Foster, S.J.; Ingham, E.; Clements, M.O. The Staphylococcus aureus Alternative Sigma Factor σ(B) Controls the Environmental Stress Response but Not Starvation Survival or Pathogenicity in a Mouse Abscess Model. J. Bacteriol. 1998, 180, 6082–6089.
52. Olivier, A.C.; Lemaire, S.; Van Bambeke, F.; Tulkens, P.M.; Oldfield, E. Role of RsbU and Staphyloxanthin in Phagocytosis and Intracellular Growth of Staphylococcus aureus in Human Macrophages and Endothelial Cells. J. Infect. Dis. 2009, 200, 1367–1370.
53. Bera, A.; Biswas, R.; Herbert, S.; Götz, F. The Presence of Peptidoglycan O-Acetyltransferase in Various Staphylococcal Species Correlates with Lysozyme Resistance and Pathogenicity. Infect. Immun. 2006, 74, 4598–4604.
54. Shimada, T.; Park, B.G.; Wolf, A.J.; Brikos, C.; Goodridge, H.S.; Becker, C.A.; Reyes, C.N.; Miao, E.A.; Aderem, A.; Götz, F.; et al. Staphylococcus aureus Evades Lysozyme-Based Peptidoglycan Digestion That Links Phagocytosis, Inflammasome Activation, and IL-1β Secretion. Cell Host Microbe 2010, 7, 38–49.
55. Kristian, S.A.; Dürr, M.; Van Strijp, J.A.G.; Neumeister, B.; Peschel, A. MprF-Mediated Lysinylation of Phospholipids in Staphylococcus aureus Leads to Protection against Oxygen-Independent Neutrophil Killing. Infect. Immun. 2003, 71, 546–549.

56. Peschel, A.; Jack, R.W.; Otto, M.; Collins, L.V.; Staubitz, P.; Nicholson, G.; Kalbacher, H.; Nieuwenhuizen, W.F.; Jung, G.; Tarkowski, A.; et al. Staphylococcus aureus Resistance to Human Defensins and Evasion of Neutrophil Killing via the Novel Virulence Factor MprF Is Based on Modification of Membrane Lipids with L-Lysine. J. Exp. Med. 2001, 193, 1067–1076.
57. Peschel, A.; Otto, M.; Jack, R.W.; Kalbacher, H.; Jung, G.; Götz, F. Inactivation of the Dlt Operon in Staphylococcus aureus Confers Sensitivity to Defensins, Protegrins, and Other Antimicrobial Peptides. J. Biol. Chem. 1999, 274, 8405–8410.
58. Li, M.; Cha, D.J.; Lai, Y.; Villaruz, A.E.; Sturdevant, D.E.; Otto, M. The Antimicrobial Peptide-Sensing System Aps of Staphylococcus aureus. Mol. Microbiol. 2007, 66, 1136–1147.
59. Kubica, M.; Guzik, K.; Koziel, J.; Zarebski, M.; Richter, W.; Gajkowska, B.; Golda, A.; Maciag-Gudowska, A.; Brix, K.; Shaw, L.; et al. A Potential New Pathway for Staphylococcus aureus Dissemination: The Silent Survival of S. Aureus Phagocytosed by Human Monocyte-Derived Macrophages. PLoS ONE 2008, 3, e1409.
60. Surewaard, B.; de Haas, C.; Vervoort, F.; Rigby, K.; DeLeo, F.; Otto, M.; van Strijp, J.; Nijland, R. Staphylococcal Alpha-Phenol Soluble Modulins Contribute to Neutrophil Lysis after Phagocytosis. Cell Microbiol. 2013, 15, 1427–1437.
61. Geiger, T.; Francois, P.; Liebeke, M.; Fraunholz, M.; Goerke, C.; Krismer, B.; Schrenzel, J.; Lalk, M.; Wolz, C. The Stringent Response of Staphylococcus aureus and Its Impact on Survival after Phagocytosis through the Induction of Intracellular PSMs Expression. PLoS Pathog. 2012, 8, e1003016.
62. Flannagan, R.S.; Watson, D.W.; Surewaard, B.G.J.; Kubes, P.; Heinrichs, D.E. The Surreptitious Survival of the Emerging Pathogen Staphylococcus Lugdunensis within Macrophages as an Immune Evasion Strategy. Cell. Microbiol. 2018, 20, e12869.
63. Boldock, E.; Surewaard, B.G.J.; Shamarina, D.; Na, M.; Fei, Y.; Ali, A.; Williams, A.; Pollitt, E.J.G.; Szkuta, P.; Morris, P.; et al. Human Skin Commensals Augment Staphylococcus aureus Pathogenesis. Nat. Microbiol. 2018, 3, 881–890.
64. Gibson, J.F.; Pidwill, G.R.; Carnell, O.T.; Surewaard, B.G.J.; Shamarina, D.; Sutton, J.A.F.; Jeffery, C.; Derré-Bobillot, A.; Archambaud, C.; Siggins, M.K.; et al. Commensal Bacteria Augment Staphylococcus aureus Infection by Inactivation of Phagocyte-Derived Reactive Oxygen Species. PLoS Pathog. 2021, 17, e1009880.
65. Davis, J.M.; Ramakrishnan, L. The Role of the Granuloma in Expansion and Dissemination of Early Tuberculous Infection. Cell 2009, 136, 37–49.
66. Drevets, D.A. Dissemination of Listeria Monocytogenes by Infected Phagocytes. Infect. Immun. 1999, 67, 3512–3517.

67. Peters, N.C.; Egen, J.G.; Secundino, N.; Debrabant, A.; Kimblin, N.; Kamhawi, S.; Lawyer, P.; Fay, M.P.; Germain, R.N.; Sacks, D. In Vivo Imaging Reveals an Essential Role for Neutrophils in Leishmaniasis Transmitted by Sand Flies. Science 2008, 321, 970–975.
68. Shi, M.; Li, S.S.; Zheng, C.; Jones, G.J.; Kim, K.S.; Zhou, H.; Kubes, P.; Mody, C.H. Real-Time Imaging of Trapping and Urease-Dependent Transmigration of Cryptococcus Neoformans in Mouse Brain. J. Clin. Investig. 2010, 120, 1683–1693.
69. Sedlyarov, V.; Eichner, R.; Girardi, E.; Essletzbichler, P.; Goldmann, U.; Nunes-Hasler, P.; Srndic, I.; Moskovskich, A.; Heinz, L.X.; Kartnig, F.; et al. The Bicarbonate Transporter SLC4A7 Plays a Key Role in Macrophage Phagosome Acidification. Cell Host Microbe 2018, 23, 766–774.
70. Xiong, Y.Q.; Fowler, V.G.; Yeaman, M.R.; Perdreau-Remington, F.; Kreiswirth, B.N.; Bayer, A.S. Phenotypic and Genotypic Characteristics of Persistent Methicillin-Resistant Staphylococcus aureus Bacteremia In Vitro and in an Experimental Endocarditis Model. J. Infect. Dis. 2009, 199, 201–208.