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REVIEW

INTERPLAY BETWEEN AUTOPHAGY AND PATHOGENIC BACTERIA: TOXINS SECRETED BY *STAPHYLOCOCCUS AUREUS* AND THEIR IMPACT ON AUTOPHAGY

Krisana Asano and Akio Nakane

Abstract Autophagy, a cellular homeostatic pathway, is emerged as an innate immune response against intracellular pathogens. It can directly eliminate invading bacteria by mediating their delivery to lysosomes. However, successful intracellular pathogens have developed mechanism(s) to escape or subvert autophagy for their intracellular niches. Studies on interplay between autophagy and intracellular pathogens are very important for understanding how infections occur. Particularly, *Staphylococcus aureus* is an important opportunistic pathogen that causes a wide range of infections. Classically, *S. aureus* is considered as an extracellular pathogen, but cumulative evidence indicates that this bacterium invades epithelial cells and replicates intracellularly which is relevant to intracellular persistence and chronic infections. A serious therapeutic problem of staphylococcal infections is caused by antibiotic resistant strains which have emerged increasingly in recent years. Thus, new insights in the strategy of *S. aureus* to interplay with autophagy is urgently required. This review highlights an impact of *S. aureus* toxins on autophagy. Alpha-hemolysin activates autophagy and prevents lysosome-autophagosome fusion, whereas toxic shock syndrome toxin-1 suppresses autophagosome formation. This opposite function indicates a complicated relationship between autophagy and intracellular adaptation of *S. aureus*. The possible effects of these toxins on *S. aureus* infections are also addressed in this review.

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Key words: *Staphylococcus aureus*; autophagy; intracellular pathogens; toxic shock syndrome toxin-1; alpha-hemolysin.

Introduction

An important strategy of numerous intracellular pathogens is the invasion of host cells in order to escape from components of the immune system. Simultaneously, cells have developed powerful mechanisms to protect themselves. They use several pathways to destroy invading pathogens such as generation of reactive oxygen species, modulation of essential cations and nutrients, and degradation by proteolytic enzymes[1]. Thus, once the intracellular pathogens have entered the host cells, they have to develop sophisticated mechanisms to overcome host cell defenses and replicate successfully[2, 3]. Some bacteria escape into the cytoplasm to avoid lysosomal killing[4, 5], whereas some other intracellular microorganisms stay inside the vacuolar phagosome, but hamper their maturation into lysosomes[6]. In the past decade, many studies demonstrated that several bacteria use a strategy to divert trafficking from the normal phagosomal pathway towards the autophagic pathway. These pathogens take control of this cell defense mechanism to obtain an advantage for their survival and replication[7, 8]. This review focuses on the interplay between *Staphylococcus aureus* and autophagy. *S. aureus* have long been considered to be an extracellular pathogen, it generally localizes and persists on human skin

Department of Microbiology and Immunology, Hirosaki University Graduate School of Medicine, Hirosaki, 036-8562 Japan

Correspondence: K. Asano
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and frequently found in nasal cavity. However, there are some data evidence that *S. aureus* can adapt into intracellular environement[9]. This bacterium produces several virulence factors to promote its infections[10]. However, the mechanism that is involved in switching of intracellular survival and autophagic interplay is still unclear. The roles of staphylococcal toxins on autophagy which contribute to *S. aureus* infection are discussed. To understand the interplay between autophagy and *S. aureus*, basic concept of autophagy including its dynamic process and interplay between autophagy with some example invading bacteria are introduced.

Autophagy and its dynamic process

Autophagy concept emerged during 1960's, when the scientists observed that cell could destroy its own contents in the enclosed membranes but only little about this organelle was known. Until early 1990's, a series excellent experiments were performed by Dr. Yoshinori Ohsumi in the University of Tokyo, Japan. He identified essential genes for autophagy and elucidated autophagy mechanism in yeast[11, 12]. He also showed that our cells use a similar machinery which leads to an understanding in the fundamental importance of this organelle. For his discoveries, he is awarded the 2016 Nobel Prize in Physiology or Medicine. Autophagy is an essential process for cell survival during nutrient starvation. This intracellular pathway delivers cytoplasmic proteins and organelles to the lysosome for degradation. Autophagy involves the formation of a double membrane around cytoplasmic substrates resulting the organelle known as an autophagosome. Under starvation, cells develop autophagy to reduce the metabolisms by reducing non-necessary cytoplasmic substances and organelles. Therefore, autophagy generally means self-eating for alive[13]. Since lysosomes break down cellular wastes in autophagosomes into the resources of metabolism, such as amino acids and free fatty acids, autophagy frequently plays a role in maintaining cellular metabolism[14]. Autophagy is also functions in the control of the quality of cellular components, therefore it is extremely important in maintaining cellular homeostasis. Although autophagy is vital for cell survival, under certain circumstances it is also implicated in cell death[15]. Thus, autophagy appears to be a double-edges sword that could be either protective or detrimental.

Currently, there are four pathways of autophagy identified by their characteristics and/or required factors. '**Macroautophagy**' is the most extensively studies and described as canonical pathway. The formation of autophagosomes in this pathway requires microtubule-associated protein light chain 3 (LC3), autophagy-related protein 5 (Atg5) and Atg7[16]. Nishida' group, showed that there exists a non-canonical pathway that leads to the formation of autophagosomes without requiring LC3, Atg5 or ATG7. This pathway relies on Ras-related protein 9 (Rab9) and refers to '**Alternative autophagy**'[17]. The other two pathways of autophagy are '**Microautophagy**' and '**Chaperone-mediated autophagy**'. However, the molecular mechanisms that mediate microautophagy and chaperone-mediated autophagy are not fully understood[16].

The canonical macroautophagy (refers to autophagy in this review) is a dynamic process including autophagy induction and initiation, elongation, and degradation (Fig. 1)[16, 18]. In each process, several proteins and protein complexes are required. Mammalian target of rapamycin (mTOR) is a main regulatory protein for autophagy induction. Under high nutrition condition, mTOR is activated. In this form, autophagy is inhibited. Under low nutrition condition, mTOR is inactivated and autophagy is induced through

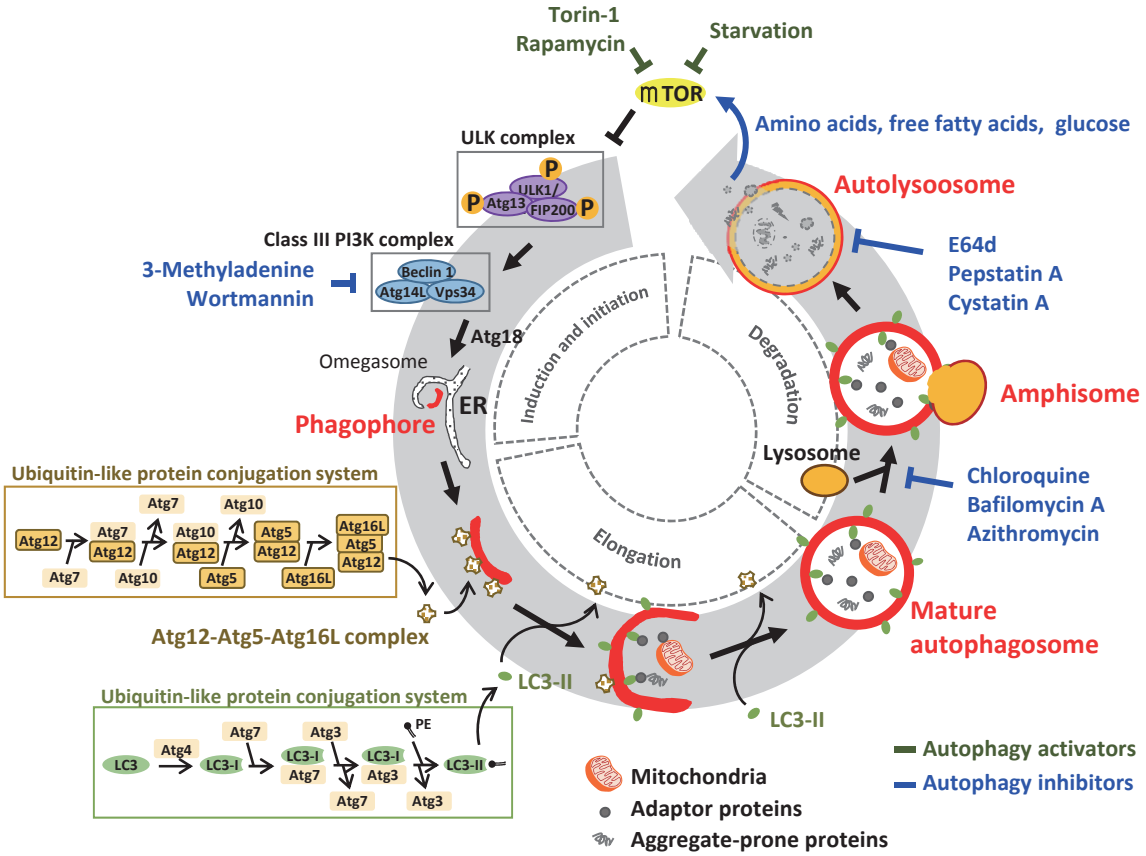

Figure 1 Schematic diagram of autophagy. Dynamic process of autophagy includes induction and initiation, elongation, and degradation. Autophagy activators and inhibitors are also indicated. mTOR; mammalian target of rapamycin, Atg; autophagy-related protein, ULK; uncoordinated-51-like kinase, PI3K; phosphatidylinositol-3 kinase, ER; endoplasmic reticulum, LC3; microtubule-associated protein light chain 3.

uncoordinated-51-like kinase (ULK) complex. Omegasome formation rising from endoplasmic reticulum is positively regulated by class III phosphatidylinositol-3 kinase (PI3K) complex. Then, isolated membrane (phagophore) is formed in the center of the ring of omegasome. In addition to the ULK and class III PI3K complexes, Atg18 is required during this process. Following the autophagosome membrane assembly, membranes continue to elongate and to sequester intracellular components. Ubiquitin-like protein conjugation systems play important roles in the elongation process[19]. Atg12-Atg5-Atg16L complexes are formed and firstly incorporate into the phagophore. In another conjugation system, Atg4 cleaves the carboxyl terminal region of LC3 immediately after synthesis, generating a soluble LC3-I. LC3-I is then conjugated to the lipid phosphatidylethanolamine (PE) to form LC3-II. The Atg12-Atg5-Atg16L complex that is already inserted into the phagophore is capable of PE binding. This capability ensures that newly converted LC3-II is incorporated into the elongating phagophore. After mature autophagosome is formed, the outer membrane of autophagosome fuses with lysosome and form the autolysosome. Lysosome is an organelle that has an acidic lumen containing over 60 different hydrolases for digesting cellular components. For the final step of autophagic degradation, the lysosomal hydrolases degrade the autophagosome-delivered contents together with its inner membrane.

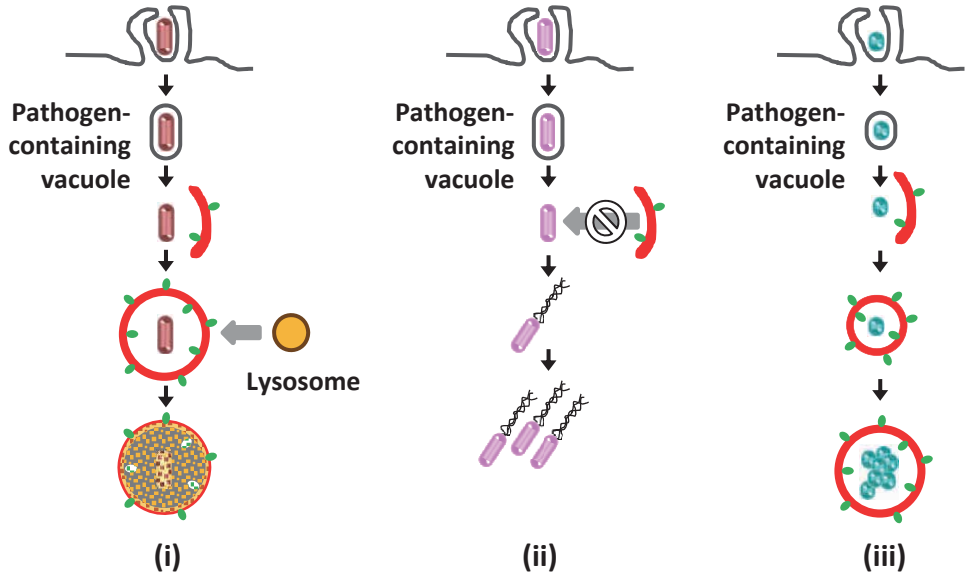

Figure 2 Complex interactions between autophagy and intracellular bacteria. (i) Bacteria that are eliminated by autophagy include *Mycobacterium tuberculosis*[24], *Salmonella enterica* serovar Typhimurium[25] and *Streptococcus pyogenes*[26]. (ii) Bacteria that are able to evade autophagy are the pathogens that can form actin rocket tail such as *Shigella flexneri*[27], *Listeria monocytogenes*[29] and *Burkholderia pseudomallei*[31]. (iii) Bacteria that have been reported to exploit autophagy for replication are *Coxiella burnetii*[32], *Legionella pneumophila*[33], *Anaplasma phagocytophilum*[34], *Yersinia pseudotuberculosis*[35] and *Brucella abortus*[36].

Autophagy has primarily been described as non-selective dynamic process to eliminate bulky garbage or to reused metabolic resources under nutrient stressed conditions. However, recent studies demonstrate that intracellular cargoes are selectively removed by autophagy. Selective substrates for autophagy degradation are mediated by adaptor proteins such as nuclear domain 10 protein 52, p62, neighbor of breast cancer susceptibility gene 1 and optineurin[20]. The selective autophagy has been described for mitochondria (mitophagy), peroxisomes (pexophagy), lysosomes (lysophagy), agggresomes (aggrephagy), liposomes (lipophagy), the nuclease (nucleophagy), ribosome (ribophagy), endoplasmic reticulum (ERphagy) and even infectious particles (xenophagy).

Autophagy and pathogenic bacteria

Xenophagy or selective autophagy that is targeted infectious particles is suggested to emerge as innate immune response pathway to combat and restrict growth of infected microorganisms. Several infectious bacteria induce the formation of ubiquitinated protein aggregates, which recognized by cargo adaptors, and are ultimately destroyed by autophagy[21, 22]. However, many intracellular bacteria have developed sophisticated mechanisms that enable them to overcome host cell defenses and replicate successfully[23]. The complex interactions between autophagy and intracellular bacteria are divided into three groups; (i) bacteria that are eliminated by autophagy, (ii) bacteria that can evade autophagy and (iii) bacteria that can exploit autophagy for replication (Fig 2).

(i) Bacteria that are eliminated by autophagy

Mycobacterium tuberculosis: After internalization *M. tuberculosis* is capable to persist within phagosomal compartment. Deretic and colleagues demonstrated that *Mycobacterium*-containing

vacuoles stimulate autophagy and colocalize with LC3 and lysosome marker, lysosomal-associated membrane protein 1. By ultrastructural analysis, the bacterium in the vacuole also shows sign of undergoing degradation. Consistent with this observation, autophagy induction by rapamycin suppresses mycobacterial survival. The data suggest that the pathogen is targeted to an autolysosome and becomes susceptible to this host-killing activity[24].

Salmonella enterica serovar Typhimurium: *S.* Typhimurium actively invades non-phagocytic cells by inducing actin rearrangement and membrane ruffling. This pathogen localizes within a membrane-bound compartment called the *Salmonella*-containing vacuoles (SCV) where the bacterium replicates. However, at early of infection, approximately one-third of intracellular population localize in damage vacuoles which are recognized by ubiquitin system and targeted by autophagy. Using Atg5$^{-/-}$ mouse embryonic fibroblasts, an increase in the number of cytosolic bacteria and enhanced replication are demonstrated, suggesting a role for autophagy in preventing the escape of the bacterium into the cytoplasm and restricting its replication[25].

Streptococcus pyogenes: *S. pyogenes* also known as Group A streptococcus (GAS) is capable of invading non-phagocytic cells by the generation of large host membrane invaginations which engulf bacteria. After internalization, GAS escapes from phagosome via the secretion of streptolysin O, a cholesterol-dependent pore forming toxin. In the cytoplasm, GAS is enwrapped by autophagic structures. More importantly, these trapped bacteria are killed by fusion of these autophagosome-like compartments with lysosomes. By comparing the bacterial number in wild type and Atg5 knockout cells, the data indicate that in autophagy-competent cells, most of GAS are killed, preventing the dissemination of the infection[26].

(ii) Bacteria that can evade autophagy

Shigella flexneri: *S. flexneri* is one of bacteria that can evade autophagy capture. This bacterium enters host cells and escapes from the phagosome into the cytosol. It produces a cell surface virulence protein, IcsA, to promote actin-based motility. However, this protein can bind Atg5 which is then targeted by autophagy for degradation[27]. To escape autophagy, *S. flexneri* produces another protein, IcsB, to bind IcsA and mask it from recognition of Atg5 and the autophagy pathway. In conjunction with autophagy, septins might restrict a minority bacterial spreading under conditions that favor anti-bacterial autophagy[28], while the majority of *S. flexneri* can escape autophagy capture in the cytosol.

Listeria monocytogenes: *L. monocytogenes* is an intracellular pathogen that can form actin-tail formation. *L. monocytogenes* escapes from phagosomes using a pore-forming toxin, listeriolysin O and two phospholipase C enzymes. In the cytosol, this bacterium uses a cell surface protein called ActA to promote intracellular motility and cell-to-cell spread. Recruitment of host actin to the bacterial surface by ActA is thought to mask *L. monocytogenes* from autophagy recognition in the cytosol[29]. In the absence of ActA, another protein, internalin K also masks intracellular *L. monocytogenes* from autophagic recognition[30].

Burkholderia pseudomallei: *B. pseudomallei* can invade epithelial cells and also survives and proliferates inside phagocytes. After phagocytosis, *B. pseudomallei* escapes from phagocytic vesicles into the cytoplasm where it replicates. In the cytoplasm, this pathogen induces actin polymerization, forming actin comets. *B. pseudomallei* also generates actin-based membrane protrusions which allow cell-to-cell spreading. Although autophagy is induced in the *B. pseudomallei*-infected cells, the survival

is not enhanced in the cells treated with autophagy inhibitors. The data suggest that *B. pseudomallei* has developed strategies to avoid killing by the autophagy pathway. Importantly, *B. pseudomallei* secretes Bop A protein which is shown to play a role in autophagy evasion[31]. A BopA deletion mutant displays a reduction of intracellular survival and an increase of LC3-colocalization. Although, protein BopA shows 23% similar to IcsB, a factor that secreted by *S. flexneri*, the molecular mechanism of BopA that permits *B. pseudomallei* to escape from autophagy remains to be elucidated.

(iii) Bacteria that can exploit autophagy for replication.

Coxiella burnetii: After internalization by the host cell, *C. burnetii* localize in early phagosomes which fuse with other vesicles to form the large parasitophorous vacuoles where this pathogen multiplies. At 5 min post-internalization, *Coxiella*-containing phagosomes are quickly recruited by LC3. However, the majority of the *Coxiella*-containing phagosomes have not acquired lysosomal enzymes immediately after LC3 recruitment. They have acquired the lysosomal enzymes at 1 h later, suggesting that there is a delay in the fusion with the lysosomal compartment. Under starvation conditions or treatment with rapamycin, an inducer of autophagy, the percentage of infected cells, the size of *Coxiella*-containing vacuoles and intracellular *C. burnetii* increase, indicating that autophagy promotes *Coxiella* replication[32].

Legionella pneumophila: *L. pneumophila* is able to multiply in aveolar macrophages, correlating to its pathogenesis. Soon after internalization, *L. penumophila* escapes phagolysosome fusion. A study demonstrated that this bacterium activates autophagy and delivers virulence factors via type IV secretory system to delay the arrival of the lysosomal enzymes. At 4-6 h after infection, the autophagosome eventually fuses with lysosome. However, the bacterium has differentiated into an acid-tolerant phenotype which is capable to replicate in the acidic compartment[33].

Anaplasma phagocytophilum: *A. phagocytophilum* is an intracellular bacterium that can avoid the degradation by lysosomal machinery of the cells. After entering into the host cells, it remains in a non-acidic vacuole that does not bind to the lysosomal markers. On the other hand, *Anaplasma* containing compartment presents features of an autophagy compartment including localization with the autophagic protein, LC3 at 48 h post-infection. These LC3-decorated vacuoles do not colocalize with the lysosomal protein, indicating that autolysosome formation is hampered. Moreover, growth of *A. phagocytophilum* is favored by the autophagy inducer, rapamycin and is arrested by autophagic inhibitor, 3-methyladenine. Thus, this pathogen is suggested to subvert the autophagy pathway leading to the biogenesis of an early autophagosome-like compartment which avoids fusion with lysosomes[34].

Yersinia pseudotuberculosis: *Y. pseudotuberculosis* is a pathogen that is able to replicate inside macrophages. It activates the autophagy pathway and is enclosed in LC3 positive double-membrane. However, the autophagosomes subverted by bacteria do not become acidified and sustain bacterial replication[35].

Brucella abortus: *B. abortus* targets to macrophages and other phagocytes to promote its infection. At 24 post infection, some bacterial cells were localized in phagosomes, whereas some others were found in autophagy compartment. When cells were incubated with autophagy inhibitors, 3-methyladenine or Wortmannin, lower bacterial yields were recovered at 24 h post infection. In contrast, in the cells subjected to autophagy induction, an increase in bacterial number was observed, indicating that autophagy plays a key role in

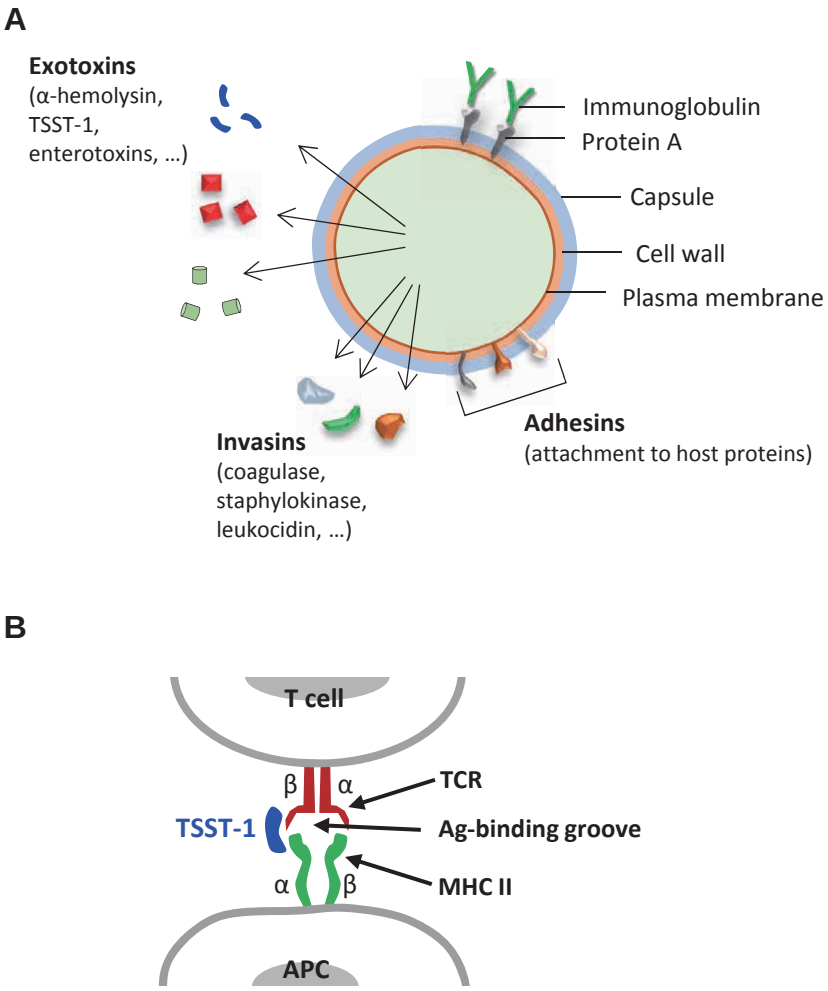

Figure 3 *Staphylococcus aureus* and its virulence factors. (A) *S. aureus* produces many potential virulence factors to promote its infections and pathogenesis including factors that inhibit phagocytic engulfment (capsule and Protein A), adhesins, invasins and exotoxins. Exotoxins that have been shown to modulate autophagy are α-hemolysin and toxic shock syndrome toxin-1 (TSST-1). (B) Superantigenic activity of TSST-1. TSST-1 crosslinks between the major histocompatibility complex class II (MHC II) on antigen presenting cell and T cell receptor bearing specific variable β element. This binding subsequently leads to a massive proliferation of T cells and uncontrolled release of proinflammatory cytokines.

the intracellular replication of *Brucella*[36].

Evidences for *S. aureus* as intracellular bacterium

S. aureus is a Gram-positive spherical bacterium that forms grape-resembling clusters. It is commonly colonized on human and animal skin, and frequently found in nasal cavity. *S. aureus* is known as a major cause a wide range of infections eventually leading to septic and toxic shock[37]. To promote its infection, this bacterium produces several virulence factors including adhesins, invasins and exotoxins (Fig. 3A)[10]. A severe clinical problem of *S. aureus* infections is an emergence of antibiotic resistant strains such as methicillin-resistant *S. aureus* (MRSA)[38]. Classically, *S. aureus* has been considered as an extracellular pathogen which is capable of tissue invading. Extracellular *S. aureus* and its secreted virulence factors are recognized by professional phagocytic cells which then an inflammatory response is stimulated. This capable of infecting resulting in cellulitis, pneumonia, osteomyelitis,

endocarditis, brain abscesses, bacteremia, and more depends on infectious tissues[39]. However, many reports have documented that *S. aureus* can invade host cells and persist intracellularly for various periods of time in culture models[9]. Some of these reports address an intracellularly phenotypic switching of small colony variant, a slow-growing subpopulation of bacteria[40]. The intracellular bacteria would provide *S. aureus* with an ideal strategy to escape from professional phagocytes and extracellular antibiotics and would promote chronic and recrudescent infections. These data correlate to some clinical cases, in which *S. aureus* infections are reported to persist asymptomatically with relapses occurring moths or even years after antimicrobial treatment and apparent cure of the infections[41].

To persist intracellularly, *S. aureus* is expected to evolve mechanism to combat with autophagy. Although *S. aureus* has been shown to induce autophagy after invasion, the interplay mechanism between *S. aureus* and autophagy is unclear. Mauthe and colleagues demonstrated that after invading into non-professional host cells, *S. aureus* cells become entrapped in autophagosome-like Atg18 positive vesicles and targeted for lysosomal degradation[42]. On the other hand, Schnaith's group reported that *S. aureus* subverts autophagy for its own replication. Eventually, the replicated *S. aureus* cells escape into the cytoplasm and induce host cell death. This capable of escape mechanism requires accessary gene regulators (*agr*) system which regulates a wide array expression of virulence factors[43]. These data suggest that interplay between *S. aureus* and autophagy is complicated. It might be varied depend on the strains of *S. aureus* and their virulence factors. Alpha-hemolysin (Hla) and toxic shock syndrome toxin-1 (TSST-1) produced have been shown to modulate autophagy.

Effect of Hla on autophagy

Hla is one of cytotoxins that functions as a homoheptameric pore-forming toxin with the phospholipid membrane. Hla is encoded by *hla* gene which is regulated under the control of *agr* system. Effect of environmental factors also appear to play a role in Hla expression such as temperature, high osmolarity, CO_2 and O_2. This altered expression does not seem to be directed by the *agr* system. After expression and secretion, the mature protein, containing 293 residues with a molecular weight of 33,000 Da, forms heptameric pore in the cell membrane which is capable of lysing eukaryotic cells, especially erythrocytes[10].

Regarding pore-forming capability of Hla, it is expected to lyse phagosomal and also autophagosomal membrane to allow the bacterial escape into the cytosol. A study demonstrated that Hla activates autophagy in a PI3K-independent mechanism[44, 45]. Hla-positive *S. aureus* localizes in LC3-positive, non-acidic and non-degradative compartment. On the other hand, Hla-negative *S. aureus* is not able to escape from phagosomal compartments. It localizes in acidic compartment unlabeled by LC3. These data suggested that Hla is required to activate autophagy and prevent autophagosomal and lysosomal fusion.

Effect of TSST-1 on autophagy

Toxic shock syndrome toxin-1 (TSST-1) is one of pyrogenic superantigens produced by *S. aureus*. TSST-1 encoding gene (*tst*) is regulated not only under *agr* system but also under other regulatory system such as ribonucleic acid III, staphylococcal accessory regulator A, staphylococcal accessory regulator B and sigma factor B. Similar to Hla, TSST-1 expression is dependent on environment factors[10]. After secretion, mature TSST-1 activates immune response mediated by non-specific interaction between the major histocompatibility complex class II on antigen

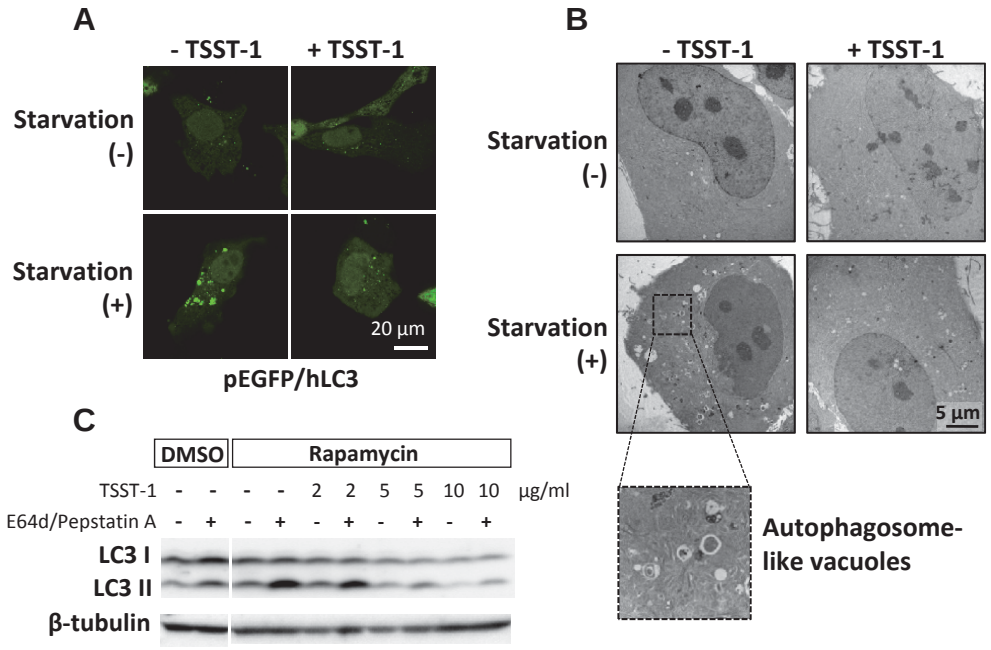

Figure 4 TSST-1 suppresses autophagy in nutrient-starved cells. (A) HeLa 229 cells were transfected with pEGFP/hLC3 plasmid to express a chimeric green fluorescent protein fused with human LC3 (GFP-LC3). Autophagy was induced upon starvation with or without addition of 10 μg/ml TSST-1. Autophagosomes represented by GFP-LC3 puncta were observed under confocal microscope. (B) Autophagy in HeLa 229 cells was induced as mentioned in Figure 4A and autophagosome-like vacuoles were observed under electron microscope. (C) Autophagy in HeLa 229 cells were induced by rapamycin with or without lysosomal protease inhibitors (E64d and Pepstatin A) and various concentrations of TSST-1. Autophagosomes represented by LC3-II accumulation was observed by Western blotting. The vehicle dimethylsulfoxide (DMSO) was used for the control. β-Tubulin was used as an internal control for protein loading. Figures are modified from Asano et al.[47].

presenting cell and T cell receptor (Fig. 3B). In addition to this superantigenic activity, TSST-1 has been shown to internalize into the epithelial cells[46] and contribute to *S. aureus* infection[47]. We have demonstrated TSST-1 does not promote *S. aureus* adhesion and invasion into the epithelial cells. On the other hand, it reduces autophagosomal accumulation in the nutrient-starved cells (Fig. 4)[48]. By using recombinant toxin and lysosomal protease inhibitors, the results suggested that TSST-1 suppresses autophagosomal formation rather enhances lysosomal fusion and degradation (Fig. 5A). This suppression is shared with a superantigenic activity-deficient mutant of TSST-1 (mTSST-1) but not with other superantigens (Fig. 5B), suggesting that this suppression is superantigenic activity-independent. The reason of autophagic suppression by TSST-1 is still elusive. We also demonstrated that TSST-1-secreting *S. aureus* suppresses autophagy in the response of infection[48]. Thus, autophagic suppression by TSST-1 might contribute in staphylococcal infection.

Interplay between *S. aureus* and autophagy

To date, the strategy of *S. aureus* to combat autophagy is unclear. Although several studies concur that autophagy is induced after *S. aureus* internalization, the interplay between *S. aureus* and autophagy is complicated. The final result of *S. aureus* infection seems to depend on strain specificity, production of virulence factors, cell types and intracellular environments. From

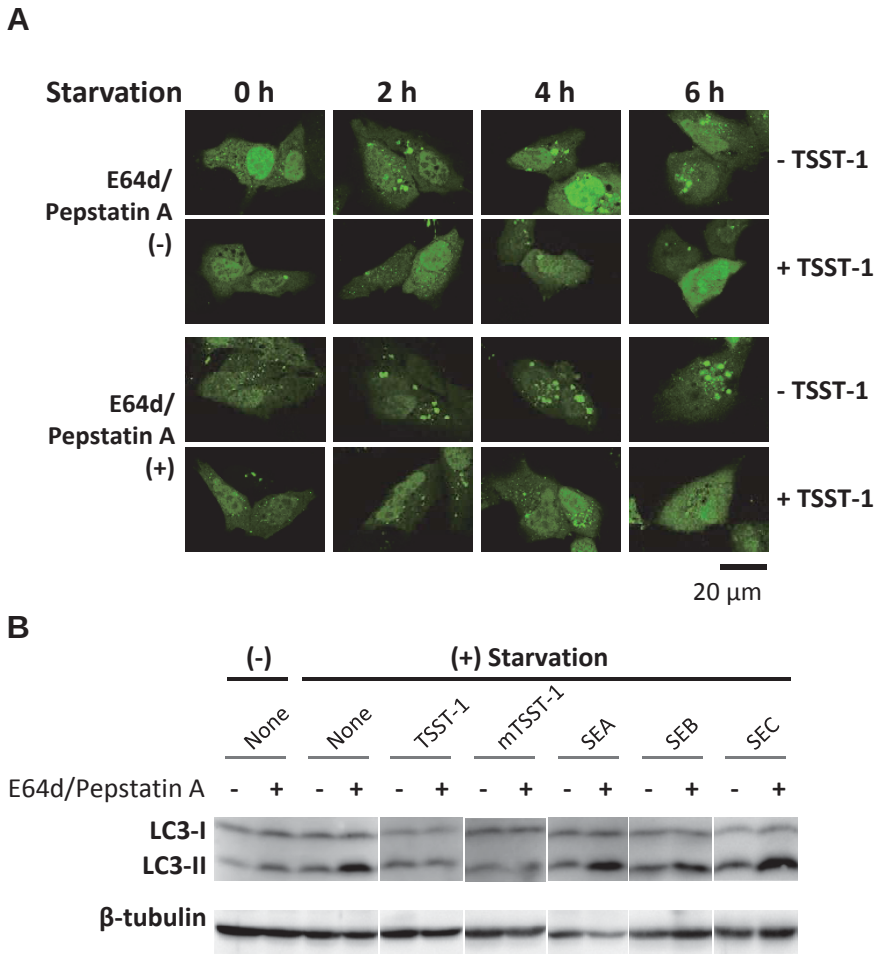

Figure 5 Mechanism of TSST-1 in autophagy suppression. (A) Autophagy flux in the GFP-LC3-expressing HeLa 229 cells was observed at 0, 2, 4, 6 h of autophagy induction. Under the condition without lysosomal protease inhibitors (E64d and Pepstatin A), GFP-LC3 puncta increased upon starvation was suppressed by TSST-1. Under the condition with E64d and Pepstatin A, GFP-LC3 puncta in TSST-1-treated cells were not restored, suggesting that TSST-1 does not enhance autophagosomal degradation. (B) Autophagy in HeLa 229 cells were induced upon starvation with or without lysosomal protease inhibitors (E64d and Pepstatin A) and 10 μg/ml of various superantigen-related toxins. Autophagosomes represented by LC3-II accumulation was observed by Western blotting. β-Tubulin was used as an internal control for protein loading. mTSST-1 is a nontoxic mutant of TSST-1, whereas SEA, SEB and SEC are staphylococcal enterotoxins exhibiting superantigenic activity. mTSST-1 exhibited a similar LC3-II suppressing effect with TSST-1, whereas other staphylococcal enterotoxins did not, suggesting that autophagy suppression by TSST-1 does not require superantigenic activity. Figures are modified from Asano et al.[47].

the accumulated data, published today, we hypothesized that a part of intracellular *S. aureus* is eliminated in autolysosomes (Fig. 6; i)[42]. For the strains that are able to produce Hla, autophagy is induced and lysosome fusion is inhibited. In this situation, *S. aureus* is able to replicate in autophagosomes, induce host cell death and is released extracellularly (Fig. 6; ii)[43, 44]. Extracellular *S. aureus* would be recognized by immune cells, which then inflammation is triggered. On the other hand, autophagosome formation is suppressed by TSST-1 producing strains. *S. aureus* may use this toxin either to avoid bacterial elimination or to reduce *S. aureus* growth and host cell death. Thus, suppression of autophagy by TSST-1 might be an alternative strategy of *S. aureus* for intracellular persistence (Fig. 6; iii)[48]. To support this assumption,

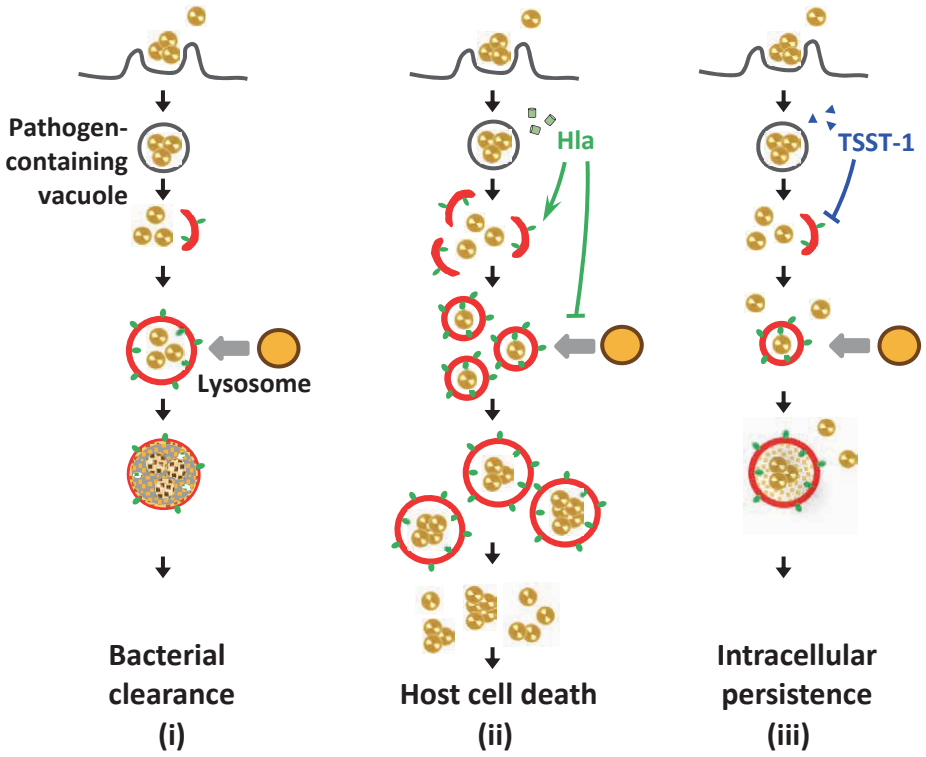

Figure 6 Possible interactions between *S. aureus* and autophagy. After internalization into the host cells, autophagy is induced. (i) *S. aureus* is entrapped in autophagosomes and eliminated in autolysosomes[42]. (ii) Autophagy induction is enhanced and lysosome fusion is inhibited by Hla. *S. aureus* subverts autophagosomes for its own replication, induces host cell death and is released extracellularly[43, 44] to promote inflammation. (iii) *S. aureus* produces TSST-1 to suppresses autophagy in order to avoid bacterial elimination in autolysosomes or to reduce *S. aureus* growth and avoid host cell death. Thus, suppression of autophagy by TSST-1 might be an alternative strategy of *S. aureus* for its intracellular persistence[47].

infectious patterns and intracellular persisting behaviors of several *S. aureus* strains including *hla*$^{-}$*tst*$^{-}$, *hla*$^{-}$*tst*$^{+}$, *hla*$^{+}$*tst*$^{-}$ and *hla*$^{+}$*tst*$^{+}$ should be compared. Moreover, expression level of Hla and TSST-1 along the stage of staphylococcal infection should be evaluated. *S. aureus* might evolve a dynamic expression level of both toxins to regulate its persistence and infections.

Conclusions

Interplay between autophagy and invading pathogens is complicated. Autophagy serves as a double-edge sword. On one hand, autophagy eliminates invading pathogens and bacterial toxins. On the other hand, successful pathogens exploit autophagy for survival and replication. *S. aureus* is a commensal bacterium that commonly colonizes on human skin and persists intracellularly. Simultaneously, *S. aureus* is an opportunistic pathogen that causes several infectious diseases. These characteristics suggest that *S. aureus* displays a dynamic relationship with autophagy. This bacterium produces several virulence factors to promote its infections. To date, at least two of them, Hla and TSST-1, have been shown to modulate autophagy. Hla activates autophagy and prevents lysosomal fusion and degradation, whereas TSST-1 suppresses autophagosome formation. It is plausible that autophagy regulation by *S. aureus* depends on a change of expression profile between Hla and TSST-1 which is relevant to the stage of persisting or

infecting behavior. Studies of interplay between autophagy and *S. aureus* are likely to yield important insights into intracellular adaptation which is a critical feature of staphylococcal persistence and chronic infectious diseases.

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