Autophagy and Ubiquitination in Salmonella Infection and the Related Inflammatory Responses

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Salmonellae are facultative intracellular pathogens that cause globally distributed diseases with massive morbidity and mortality in humans and animals. In the past decades, numerous studies were focused on host defenses against Salmonella infection. Autophagy has been demonstrated to be an important defense mechanism to clear intracellular pathogenic organisms, as well as a regulator of immune responses. Ubiquitin modification also has multiple effects on the host immune system against bacterial infection. It has been indicated that ubiquitination plays critical roles in recognition and clearance of some invading bacteria by autophagy. Additionally, the ubiquitination of autophagy proteins in autophagy flux and inflammation-related substance determines the outcomes of infection. However, many intracellular pathogens manipulate the ubiquitination system to counteract the host immunity. Salmonellae interfere with host responses via the delivery of ~30 effector proteins into cytosol to promote their survival and proliferation. Among them, some could link the ubiquitin-proteasome system with autophagy during infection and affect the host inflammatory responses. In this review, novel findings on the issue of ubiquitination and autophagy connection as the mechanisms of host defenses against Salmonella infection and the subverted processes are introduced.

Keywords: Salmonella, autophagy, ubiquitination, effector protein, inflammation

INTRODUCTION

According to the figures released by the Centers for Disease Control and Prevention, one million foodborne illnesses have been caused by Salmonella in the United States annually, with 19,000 hospitalizations and 380 deaths (https://www.cdc.gov/salmonella/). Salmonellae most often cause gastroenteritis and typhoid fever ranging from mild to severe systemic infections that might be potentially life threatening. Salmonella enterica serovar Typhimurium (S. Typhimurium) and Typhi are the respective pathogens in Salmonella family that cause this debilitating condition. Additionally, increasingly reports demonstrated that Salmonella infection is related to many other diseases such as Gallbladder cancer (GBC), thus raising a worldwide major public health concern (Scanu et al., 2015). Salmonella enterica are transmitted by the fecal-oral route through contaminated food or water. The bacteria in the small intestine adhere to the mucosa and then preferentially invade the epithelial cells of the terminal ileum. Pathogens encounter the phagocytes including dendritic cells and macrophages after traversing the epithelial layer. They are ingested and can survive in the SCVs (Salmonella-containing vacuoles) of phagocytes. Moreover, the invading pathogens could
spread to the mesenteric lymph nodes, and some disseminate to the reticuloendothelial cells of the extra-intestinal organs such as liver and spleen causing systemic infection (Keestra-Gounder et al., 2015).

Autophagy is a preserved process in eukaryotic cells that delivers cytoplasmic contents to the lysosome for degradation. The cytoplasmic materials such as damaged organelles, misfolded proteins, or intracellular microbes were engulfed by an isolation membrane (phagophore), which elongates to form a double-membraned vacuole (autophagosome), followed by the fusion with lysosome to form an autolysosome, in which the enclosed materials degraded to maintain cellular homeostasis (Deretic, 2011). Autophagy can be classified into selective and non-selective autophagy according to the degraded substance, and the selective clearance of pathogens by autophagy is regarded as xenophagy (hereafter referred as autophagy). Additionally, autophagy is a dynamic process with numerous ATG (autophagy related) proteins and autophagy adaptors involved, the host autophagy flux could be influenced by the interaction between pathogens and them. Once infected with Salmonella, autophagy can be induced rapidly which plays a pivotal role in the elimination of bacteria and the process of autophagy can even affect the following innate and adaptive immune responses to pathogens (Gomes and Dikic, 2014). Chaperone-mediated autophagy (CMA) is involved in transportation of specific cytosolic proteins to lysosomes for degradation. Interesting, CMA does not participate in the clearance of Salmonellae, on the contrary, nutrients in favor of intracellular Salmonellae growth can be supplied by CMA-dependent pathway (Singh et al., 2017).

Ubiquitination is an enzymatic cascade reaction by which ubiquitin (Ub) is covalently bound to protein substrates, mediated by E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 (Ub ligase enzyme), and this process can be reversed by deubiquitinases (DUBs). Ubiquitination is one of the pivotal “eat-me” signals, initiating the process of autophagy. Ub contains eight distinct chains, seven are lysine residues including K6, 11, 27, 29, 33, 48, and 63. The carboxy-terminal glycine of Ub attaches to an active-site cysteine of E1 through a reactive thioester bond. The activated Ub is transferred to the E2 by an analogous reaction, and the E3 catalyzes the attachment of the Ub to a lysine in the target protein. The existence of mono-Ub and poly-Ub was determined by the relative proportion between the E3 and the target protein. Besides, a special linear M1 (methionine)-ubiquitination chain was identified as the eighth Ub chain, which was generated by the formation of a peptide bond between the aminoterminal methionine residue of the preceding Ub molecule and the carboxy-terminal glycine (Walczak et al., 2012). These distinct Ub chains have different effects on the function of protein substrates. In terms of autophagy, different Ub chains show different affinities for autophagy receptors. For example, ubiquitination of the autophagy receptor p62 (the Ub sensor SQSTM1), was suggested to display a preference for K63 chains over K48 (Gomes and Dikic, 2014). The Ub ligase Smurf1 plays a role in autophagy of intracellular bacteria such as Mycobacterium tuberculosis (M. tuberculosis, Mtb) and Listeria monocytogenes (L. monocytogenes). Smurf1 recruits the proteasome, K48 Ub chain, and the autophagy machinery components to Mtb therefore restricts the replication of bacteria in macrophages both in vitro and in vivo. It was noticeable that the function of Smurf1 in autophagy requires K48-linked ubiquitination rather than K63 (Franco et al., 2017). The ubiquitination pathway and involvement of different enzymes in ubiquitination cascades are capable of regulating the metabolism and function of proteins as well as the inflammation, immunity and so forth. Eldridge et al. uncovered that the UBE2L3 is a E2 Ub conjugating enzyme which plays an essential role in inflammation. It can be targeted by inflammasomes and leads to the activation of caspase-1 as well as the production of mature IL-1β (Eldridge et al., 2017). DUBs that are specific toward topologies of different Ub chains can remove Ub moieties and shape the proteins fate. Small molecule inhibitors with DUB activity such as USP5 and UCH-L5 were identified to regulate the assembly and activation of inflammasome (Lopez-Castejon et al., 2013; Kummari et al., 2015).

In addition, ubiquitination is one of the post translational modifications (PTMs), which modifies virulence factors such as Type III secretion system (T3SS) effector proteins to manipulate its interplay with host cells. Reciprocally, some effector proteins act as Ub ligase enzyme or DUBs affecting autophagy flux or inflammatory responses. Therefore, targeting ubiquitination of proteins and ubiquitination pathways might be helpful for the resistance against Salmonella infections through modulating the process of autophagy and inflammation (Salomon and Orth, 2013). As the host defenses, autophagy and inflammation both play an essential role in resisting pathogens and they have some close ties with each other. On one hand, mediators of inflammation including innate immune receptors, inflammatory cytokines and inflammation-related transcription factors can regulate autophagy. On the other hand, autophagy can regulate inflammatory responses by modulating the activation of inflammasomes, the polarization of immune cells and the secretion of inflammatory cytokines.

To counteract the host immunity, Salmonella can subvert the Ub and autophagy pathways by delivery of several bacterial effectors into the cytosol to evade the host defenses. In this review, we discuss mechanisms by which Ub and autophagy work on the resistance against Salmonella infections as well as the related inflammatory responses. Meanwhile, we address how Salmonellae escape from either autophagy or inflammation by disturbing the process of ubiquitination.

**UBIQUITIN-DEPENDENT AUTOPHAGY IN THE ELIMINATION OF SALMONELLA**

Autophagy plays an essential role in host responses to various environmental stimuli as a housekeeping, especially the invasion of bacteria. The clearance of Salmonellae by autophagy is a kind of selective autophagy that is involved in the process of recognition, recruitment, and elimination (Gomes and Dikic, 2014). Ubiquitination plays a crucial role in bacterial recognition and targeting, and even modifies the core autophagy components, regulating the clearance of pathogens by autophagy.
flux (Grumati and Dikic, 2017). Upon exposure to cytoplasm, Salmonellae are ubiquitinated immediately and then shipped to the autophagy receptors as cargoes. LC3, the autophagy modifier (microtubule associated protein 1 light chain 3, MAP1LC3, abbreviated as LC3 henceforth) is located on the autophagosomal membrane. LC3-interacting region (LIR) motif is a domain contained in the autophagy receptors p62, NDP52 (nuclear domain 10 protein52), and OPTN (Optineurin) (Gomes and Dikic, 2014). All the receptors also hold a similar domain that functions as the Ub-binding. The pathogens interact with LIR through Ub, inducing the formation of phagophore, then the subsequent formation of autophagosome and the degradation of bacteria in autolysosome (Figure 1). Accordingly, ubiquitination is indispensable in clearance of bacteria by autophagy. However, pathogens evolve numerous strategies to subvert autophagy to protect them from elimination.

As facultative intracellular bacteria, Salmonellae could reside in both phagocytes and non-phagocytic cells such as epithelial cells. T3SSs are used by bacteria to export proteins from bacterial cytosol into eukaryotic cells. These bacterial effector proteins

![Figure 1](https://www.frontiersin.org/articles/10.3389/fcimb.2018.00078/full#tab1)
involving in the invasion of the epithelial barrier and the pathogenesis of infection. The mechanism of T3SS delivery is a contact-dependent process and highly conserved, characterized by the formation of pores, or translocon on cytomembranes of host cells when bacteria contact with them and through which the effector proteins are delivered into the host cell (Song et al., 2017). S. Typhimurium possesses two distinct T3SSs, located on Salmonella Pathogenicity Islands 1 and 2 (SPI1 and SPI2), called T3SS-1 and T3SS-2, respectively (Burkinshaw and Strynadka, 2014). After invading the host cells, Salmonella reside and replicate inside the modified phagosomes that termed SCVs. It seems to be helpless for the host to respond to the Salmonellae within SCVs. Nevertheless, the needle-like apparatus T3SS-1 can cause damage to the SCVs, which gives rise to multiple consequences for the pathogen. The majority of invading S. Typhimurium enter the SCVs for replication, while other portions can damage the SCVs by T3SS (Owen and Casanova, 2015). Subsequently, a portion of S. Typhimurium manages to escape from the SCVs and proliferates in the cytosol. These bacteria that either egress into cytosol or remain residing in damaged SCVs are rapidly ubiquitinated and then targeted by autophagy receptors (p62, OPTN, NDP52) to the autophagosome which ultimately fuses with the lysosome in which degradation of the bacteria occurred (Figure 1) (Birmingham and Brumell, 2006; Gemma et al., 2011). SifA is a T3SS-2 effector protein with N and C-terminal domain supporting Salmonella virulence. Its N-terminal domain is critical for maintaining the integrity of SCVs by inducing tabulation of the SCV and binding the mammalian kinesin-binding protein SKIP, which is critical for bacterial proliferation and evading inflammation mediated by caspase-11. Its C-terminal domain possesses the activity of guanine nucleotide exchange factor contributing to SifA virulence independent of its N-terminal domain (Beuzón et al., 2000; Ohlson et al., 2008; Aachoui et al., 2013a; Zhao et al., 2015). SifA seems essential against the initiation of autophagy to some extent due to its role in maintaining the integrity of SCVs. As a modulator of phosphoinositide 3-phosphate (PI(3)P) levels on early and recycling endosomes by dephosphorylation, the phosphoinositide 3-phosphatase myotubularin 4 (MTMR4) plays a role in survival of S. Typhimurium. Additionally, the regulation of PI(3)P is also requisite for the stability and integrity of SCVs, which may modulate the process of autophagy (Teo et al., 2016).

Undoubtedly, there are more than one possible autophagy-dependent pathway of pathogen degradation. Either Ub-dependent or -independent mechanism is involved in pathogen targeting. In particular, Ub, a well-known signal for the degradation of polypeptides in the proteasome, together with autophagy are of great importance for bacterial degradation. Certainly, both the Ub-proteasome system and autophagy play crucial roles in Salmonella infection. It has been found that a portion of intracellular S. Typhimurium exposed to the cytoplasm is rapidly ubiquitinated, leading to the recruitment of several autophagy receptors, including NDP52 and p62, as well as the TANK-binding kinase 1 (TBK1) (Rogov et al., 2013). Analysis using immunofluorescence microscopy shown that cytosolic Salmonellae are recognized by p62, NDP52, OPTN, Nap1 (NF-κB activating kinase-associated protein 1), Sinbad (also known as TBKBP), and TBK1 (TANK binding kinase1) (Ivanov and Roy, 2009). p62 was identified as the first mammalian autophagy adaptor, and it participates in many autophagy processes, including the selective degradation of cytosolic proteins and the clearance of intracellular pathogens. p62 has two domains, the Ub-associated (UBA) domain of p62 binds Ub-coated Salmonellae and LIR motif of p62 binds LC3, through which p62 facilitates the recognition of Salmonellae by autophagy (Fujita and Yoshimori, 2011). p62 also involves in a novel pathway regulating autophagy by inducing autophagosome biogenesis. p62 can promote the delivery of itself and cargoes to the autophagosome through its ZZ binding domain (Figure 1). In addition to the regulating of autophagy process, p62 may also mediates the crosstalk between autophagy and the Ub-proteasome system (Cha-Molstad et al., 2017). NDP52 is another receptor that recognizes ubiquitinated Salmonellae and is associated with bacterial clearance via the autophagy pathway (Ivanov and Roy, 2009). NDP52 binds the adaptor proteins Nap1 and Sinbad and recruits TBK1 nearby the cytosol-exposed bacteria (Ishimura et al., 2014). Thurston et al. observed that the host proteins Nap1 and Sinbad colocalize with ubiquitinated Salmonellae, which may restrict the growth of Salmonellae in eukaryotic cells. NDP52 also binds LC3, and the silencing of NDP52 impairs the clearance of Salmonellae by autophagy (Figure 1) (Thurston et al., 2009). Galectin-8, a cytosolic lectin, is a bacterial restriction factor that can induce NDP52-mediated autophagy. Upon the damage of SCVs, the following exposed host glycans recruit and bind abundant galectin 8, which could be recognized by NDP52 and then the NDP52-galectin-8 complex targets the Ub-associated S. Typhimurium to initiate autophagy (Figure 1) (Thurston et al., 2012). TANK-binding kinase 1 (TBK1) is a member of the inhibitor of nuclear factor κB kinase (IKK) family and participates in innate immune responses. The activity of TBK1 is required to induce the innate immune responses against viral infection through enhancing the expression of type I interferons and other antiviral proteins (Weidberg and Elazar, 2011). Radtke et al. reported that TBK1 restricts the replication of cytosolic Salmonellae, which is distinct from its role in virally infected cells (Radtke et al., 2007). On one hand, TBK1 confines the expression of the water channel aquaporin-1 that is crucial for the integrity of SCVs, which results in Salmonellae exposed to the cytosol followed by the accumulation of poly-ubiquitinated proteins on the surface of bacteria, and then facilitates the elimination of Salmonellae by autophagy (Ivanov and Roy, 2009). On the other hand, TBK1 phosphorylates OPTN at Ser177, thereby leading to enhanced affinity for LC3. In addition, it demonstrates that TBK1 can also phosphorylates S473 and S513 in OPTN to facilitate Ub chain binding (Heo et al., 2015). It has been revealed that TBK1 is indispensable for the recruitment of WIP12, a PI(3)P-binding component of upstream autophagy (Thurston et al., 2016). OPTN also has two domains, UBA and LIR motif (Figure 1). OPTN can bind Ub-coated cytosolic Salmonellae and the depletion of OPTN in HeLa cells promotes bacterial replication, suggesting that OPTN mediates clearance of pathogens by autophagy. Beyond its function as an autophagy receptor, OPTN is closely related to maturation of
autophagosomes to autolysosomes, indicating that it promotes the autophagy in antimicrobial immunity (Thurston et al., 2016). Weidberg et al. verified that NDP52 and OPTN function together to mediate Salmonella degradation. It is possible that NDP52 functions upstream of OPTN and locally activates TBK1 to enable recruitment of LC3 by OPTN (Weidberg and Elazar, 2011). NDP52 plays a dual function during autophagy. It can target bacteria to nascent autophagosomes at the initiation of autophagy and ensure subsequent pathogen degradation by regulating maturation of autophagosome, respectively (Verlhac et al., 2015). Intriguing, as has been mentioned above, both OPTN and NDP52 are able to facilitate autophagosome maturation, which depend on myosin V1 adaptor proteins, accelerating the autophagy-dependent clearance of S. Typhimurium (Tumbarello et al., 2015).

Considering that the autophagy process is associated with cytoskeletal machinery and integrity of the membrane structure, the dynein clusters may also play essential roles in antimicrobial autophagy flux (Sotthibundhu et al., 2016). SifA-SKIP interaction modulates the activity of the molecular motor kinesin, which may be the fundamental to the formation of autophagosomes. Contradictory to the viewpoint mentioned above that SifA is helpful for evading activation of autophagy, it may have facilitation toward autophagy flux. Furthermore, a latest study shows that Dynein motors physically cluster into lipid rafts on the membrane of a phagosome along with which matures inside the cell (Rai et al., 2016). The geometric organization in clusters makes many motors united together to drive transportation of the phagosome, and promote phagolysosome fusion. Therefore, the activity of the molecular motor kinesin is also critical for elimination of Salmonella by accelerating the formation of autophagosomes.

It was demonstrated that E3 Ub ligases control substrate selectivity in the ubiquitination cascades. Recently, RNF166 are identified as E3 Ub ligases that are required for adaptor protein recruitment and LC3-bacteria colocalization. RNF166 has a dual role in controlling the recruitment of Ub as well as p62 and NDP52 to bacteria. Though there are no valid explanations for this phenomenon, it was speculated that different E3 ligases promote different Ub chain linkages (Heath et al., 2016). The HECT E3 Ub ligase NEDD4 is identified as an LC3-interactive protein, regulating autophagy through a conserved LIR domain (Sun et al., 2017). As adaptor protein, p62 is one of the core autophagy components and the modulation of p62 by ubiquitination may determine the process of autophagy. The ubiquitination of p62 can disrupt dimerization of its UBA domain and then its ability to recognize poly-ubiquitinated cargoes was accessible to selective autophagy. The interaction between Ub conjugating enzyme UBE2D2/3 and p62 is essential for the upregulation of Ub homeostasis (Ub+), which promotes recognition of poly-ubiquitinated cargoes and autophagy flux (Peng et al., 2017). The ubiquitination of UBA domain of p62 by the Keap1/Cul3 complex augments its activity of sequestering ubiquitinated cargoes and recruits them to the growing autophagosome (Lee et al., 2017). The influence of p62 ubiquitination on autophagy flux related to Salmonella infection has not been thoroughly studied previously.

These Peng and Lee’s remarkable findings shed new light on the predominant effect of ubiquitination on autophagy flux.

Several mechanisms of antimicrobial autophagy are described to impact directly or indirectly on Salmonella clearance. Initiation of autophagy is determined by various autophagy adaptors (p62, NDP52, OPTN etc.) and the affinity of binding partners (galectin 8, TBK1, and LC3). Meanwhile, the maturation of autophagosome is implicated in this process. In order to illuminate the Ub-dependent autophagy in the elimination of Salmonella, attention should be paid to the ubiquitination as well as the detailed process of autophagy.

**SALMONELLA T3SS EFFECTOR PROTEINS AFFECT THE ANTIBACTERIAL INFLAMMATION AND AUTOPHAGY BY TRIGGERING THE UBIQUITIN PATHWAY**

Salmonella T3SSs secrete and deliver effector proteins that are closely related to pathogenesis, including invasion of the host cells, pyroptosis, intracellular survival, and evasion of host immune responses. Some effectors, such as SipA, SipC, SopB, SopD, SopE, and SopA, participate concertedly in the invasion process and bacterial internalization (Rossignol et al., 2014). Some other effectors contribute to the modulation of the inflammatory responses, most of which are relevant to ubiquitination pathway or Ub. Several SPI-1 and SPI-2 effectors interfere directly with Ub-related processes, such as E3 ligases SopA, SspH1, SspH2, and SlrP (Ria et al., 2009), and the DUB Ssel (Figure 2) (Rytkönen et al., 2007). Furthermore, SPI-1 T3SS effectors SopA, SopE, and SopB/SigD are all translocated and then ubiquitinated, some of which can work together with Ub to interfere with the immune responses.

SopA mimics mammalian HECT E3 Ub ligase and preferentially utilizes the host UbcH5a, UbcH15, and UbcH7 as E2 Ub conjugating enzymes, which are involved in stimulation of inflammation (Diao et al., 2008). It has been reported that SopA interacts with human RMA1 (HsRMA1), a RING finger family E3 Ub ligase, promoting the escape of Salmonellae from the SCVs. In an HsRMA1-dependent manner, sopA mutant escapes less frequently from the SCVs into cytosol than wild-type Salmonella does (Zhang et al., 2006). SopA can stimulate innate immune signaling by targeting Tripartite motif (TRIM) 56 and TRIM65, two host E3 Ub ligases (Kamanova et al., 2016). As an HECT-like E3 Ub ligase, SopA can also ubiquitinate TRIM56 and TRIM65, resulting in their proteasomal degradation during infection (Figure 2 and Table 1) (Fiskin et al., 2017). The Salmonella effectors SspH1 and SspH2 belong to the NEL family of proteins that mediate E3 Ub ligase activity (Table 1). These effectors mimic eukaryotic proteins to subvert the immune responses. SspH1 acts as an E3 Ub ligase, and interacts with the mammalian protein kinase PKN1 to inhibit the NF-κB signaling pathway (Keszei et al., 2014). It was shown that SspH2 could modulate innate immunity in both mammalian and plant cells using model systems in a cross-kingdom approach. In
Salmonella T3SS effector proteins that interact with the eukaryotic ubiquitin system. Some effectors act as E3 ligase interacting with host proteins to inhibit NF-κB pathways, such as SopA, SlrP, SspH1, and SspH2. The others serve as DUB to play a role on the responses to infection through Ub pathway. Ssle is the best example. GogB and AvrA are effectors that interact with the host Ub pathways following diversity mechanism to impact the immune system. SseK and SteD can affect host immune responses by interacting with E3 Ub ligase TRIM32 and MARCH8, respectively. Furthermore, SopB, SopA, and SopE could also be self-ubiquitinated to influence the antimicrobial process. However, several effectors with ubiquitination sites remain to be determined.

mammalian cell culture, SspH2 significantly enhanced Nod1-mediated IL-8 secretion when transiently expressed or bacterially delivered (Table 1) (Bhavsar et al., 2013). In a nucleotide-binding leucine-rich repeat receptor (NLR) model system, SspH2 causes phenotypic modifications, which require its catalytic E3 Ub ligase activity and interaction with the conserved host protein NLR co-chaperone SGT1 (Bhavsar et al., 2013). SlrP is another E3 Ub ligase that employs thioredoxin as a substrate. Thioredoxin plays an important role in redox regulated signaling events and participates in numerous physiological processes (Figure 2 and Table 1) (Cordero-Alba and Ramos-Morales, 2014). SlrP contains 10 copies of a leucine-rich repeat (LRR) signature and is translocated into host cells by both T3SS-1 and T3SS-2, which influences inflammation responses in the early and late stages of infection (Table 1). The IpaH family effectors were mainly characterized in Shigella, however, these proteins are widely conserved among animal and plant pathogens, including Salmonella effectors SspH1, SspH2, and SlrP. They are characterized as novel E3 ligase (NEL) enzymes, which recruit host substrates for ubiquitination through a LRR domain (Rohde et al., 2007; Keszei et al., 2014). Shigella effectors IpaH possess the activity of E3 ligase activity, target and inhibit NF-κB signaling by manipulating the host Ub system and leading with downregulation of host inflammatory responses. In the same way, we speculate that Salmonella T3SS effectors SspH1, SspH2, and SlrP may also interact with host Ub to regulate the inflammatory responses (Ashida et al., 2015).

The virulence-associated effector protein AvrA of Salmonella is a Ub-like acetyltransferase/cysteine protease, which interferes with the first line of immune defense. AvrA interferes with the NF-κB pathway by removing Ub from Ub-IκBα or via the acetylation of specific mitogen-activated protein kinase kinases (MAPKKs). AvrA mitigates IL-8 production and thereby inhibits the inflammatory responses of the host against infectious agents (Figure 2 and Table 1) (Giacomodonato et al., 2014). GogB is a substrate of both T3SS-1 and T3SS-2 in some Salmonella strains. GogB has an N-terminal LRR domain similar to those of LRR-containing Salmonella effectors SspH1, SspH2, and SlrP (Quezada et al., 2009). GogB inhibits IL1 production and interferes with NF-κB activation by inhibiting IκBα degradation through its interaction with the Skp1-Cullin1-F-box (SCF) E3 Ub ligase, leading with a limited activation of innate immune defenses in the host (Figure 2). Anti-inflammatory properties of GogB are important for the bacteria to reach optimal infection density in host tissues and to limit the tissue damage associated with a prolonged active inflammatory responses. These results suggest that GogB may play an essential role in downregulating the host inflammatory responses during infections (Pilar et al., 2012). In addition, the T3SS effector SseK3 is relevant to Salmonella infection through a novel molecular interaction with an E3 Ub ligase, TRIM32 (Yang et al., 2015). Like other E3 Ub ligases, TRIM32 is able to regulate its own activity by auto-ubiquitination. The relevance of the SseK3-TRIM32 does play a critical role in Salmonella infections and the modulation of NF-κB activation via ubiquitination pathway, even though the
### TABLE 1 | Salmonella T3SS effector proteins that interact with the eukaryotic ubiquitin system.

| Effector | Activity | Interacting partner | Consequence of the interaction |
|----------|----------|---------------------|------------------------------|
| SopA     | HECT-like E3 ubiquitin ligase | Human RMA1 (HsRMA1) | Promote the escape of Salmonella from the SCVs |
| SspH1    | Mammalian protein kinase PKN1 | E3 ubiquitin ligaseTRIM56 and TRIM65 | Result in their proteasomal degradation |
| SspH2    | E3 ubiquitin ligase | NOD1 | Inhibit the NF-κB pathway |
| SlpP      | E3 ubiquitin ligase | Thioredoxin | Enhance IL-8 secretion |
| AvrA      | Ubiquitin-like acetyltransferase/cysteine protease | Skp1-Cul1-F-box (SCF) E3 ubiquitin ligase | Regulate signaling events and physiological processes |
| GogB      | E3 ubiquitin ligase | E3 ubiquitin ligase TRIM32 | Inhibit IL-1 production and interferes with NF-κB activation |
| SseK3     | E3 ubiquitin ligase | E3 ubiquitin ligase MARCH8 | Modulate NF-κB activation |
| SteD      | Deubiquitinase | Ubiquitin | Inhibit dendritic cell-mediated activation of T cells |
| SseL      | Ubiquitin | Unknown | Interfere with the NF-κB pathway |
| SopA      | Self-ubiquitination | Unknown | Inhibit autophagy by reducing ubiquitinated aggregations |
| SopB      | Undetermined | Undetermined | Degradate through the HsRMA1-mediated ubiquitination pathway |
| SopE      | Undetermined | Undetermined | Promote neutrophil accumulation at the site of infection |
| SipA      | Undetermined | Undetermined | Degrade through proteasome-mediated pathway |
| SipB      | Undetermined | Undetermined | |
| SipC      | Undetermined | Undetermined | |
| SopE2     | Undetermined | Undetermined | |

specific mechanism is yet unclear (Figure 2). MHC class II molecules play a major role in adaptive immune responses by presenting antigens to the CD4 restricted T cells. Salmonellae are able to interfere with the expression of MHC in both professional and non-professional antigen presenting through ubiquitination of HLA-DR in the cell surface, thus depressing the MHC antigen presentation. And this is dependent on T3SS-2 (Lapaque et al., 2009). The T3SS-2 effector SteD was proved to be required and sufficient for suppressing the activation of T cell during Salmonella infections. This process is also closely related to the ubiquitination pathway. SteD binds surface-localized mature MHC class II (mMHCII) as well as the host E3 Ub ligase MARCH8 (membrane associated ring-CH-type finger 8). It employs MARCH8 to promote mMHCII ubiquitination and surface depletion, leading to inhibition of dendritic cell-mediated activation of T cells (Figure 2 and Table 1) (Bayer-Santos et al., 2016). In addition to their action in adaptive immune responses, endosomal MHC class II (MHCII) molecules were proved to regulate innate immunity via sharp tuning the TLR4 signaling pathway. However, MARCH1 E3 Ub ligase exerts an MHCII-independent effect that accommodating the innate immunity based on ubiquitination (Galbas et al., 2017). Although AvrA, GogB, SseK3, and SteD are the effectors without enzymatic activity of ubiquitination, they can affect the inflammatory responses by degrading Ub or interacting with different E3 Ub ligases.

Intravacuolar S. Typhimurium induces SPI-2 T3SS-dependent ubiquitination of protein aggregations during infection, the formation of ubiquitinated aggregations are subsequently subjected to autophagy (Szeto et al., 2006). However, this effect may be counteracted by the SPI-2 T3SS DUB SseL, which has a preference for K63-linked chains and contributes to macrophages death (Coombes et al., 2007; Rytkönen et al., 2007; Le Negrate et al., 2008). SseL DUB activity reduces the Ub level of p62 and LC3 in SCV-associated aggregates, suggesting that SseL reduces autophagy flux in infected cells. Previous studies demonstrated that SseL may regulate antigen presentation in infected macrophages and dendritic cells, contributing to the virulence of S. Typhimurium in mice and reducing the innate immune responses in vivo. Replication of SseL deletion-mutant Salmonella in macrophages is significantly decreased compared with that of wild-type bacteria. Overall, it could be concluded that SseL decreases autophagy flux and favors intracellular Salmonella replication by interfering with ubiquitination pathway (Mesquita et al., 2012).

Beyond that some T3SS effectors have a vital influence on Salmonella infection by interacting with ubiquitination pathway mentioned above, the T3SS effectors SopA, SopE, and SopB are able to be self-ubiquitinated (Figure 2 and Table 1). SopA and SopE are degraded through the HsRMA1-mediated ubiquitination pathway and proteasome-mediated pathway, respectively (Zhang et al., 2005). Upon infection, SopB is ubiquitinated in the host cell and then required for neutrophil accumulation at the site of infection in the intestine. Ubiquitination of SopB is necessary for appropriate vesicle trafficking of the SCVs, and it activates RhoG to remodel actin, resulting in membrane ruffles. Self-ubiquitination of SopB leads to recruitment of Rab5 to the SCVs, which normally participates in the maturation of phagolysosomes (Patel et al., 2009). Ruan et al. showed that UbcH5C/TRAF6 is a Ub ligase involved in the ubiquitination of SopB (Ruan et al., 2014).

Many ubiquitination sites of different SPI-1 effectors were detected by quantitative ubiquitination site profiling using diGly proteomics, such as SipA, SipB, SipC, SopE2, SlpP,
SopA, GogB, SopB, and SopE (Fiskin et al., 2016). The role of the effectors SptP, SopA, GogB, SopB, and SopE have been clearly identified during Salmonella infections by the characterization of their ubiquitination sites and their interacting partners, while the role of the rest effector proteins such as SipA, SipB, SipC, and SopE2 remains to be determined (Figure 2 and Table 1). Ubiquitination is a process involves in various antimicrobial mechanisms such as autophagy and inflammation as mentioned above, the novel identified effectors with ubiquitination site may play potential roles in Salmonella infection.

THE INFLAMMATORY RESPONSES TO SALMONELLA INFECTION AND ITS INTERACTION WITH AUTOPHAGY AS WELL AS UBIQUITINATION

In response to Salmonella infection, inflammation of the host is stimulated by the production of the pro-inflammatory cytokines and the activation of inflammatory caspases. Caspase-1 and caspase-11 in mice, and caspase-1, caspase-4, and caspase-5 in humans are the inflammatory caspases, and they are activated through the stimulation of either the NLRC4 or NLRP3 inflammasome (Martinon and Tschopp, 2007). Apart from its direct antimicrobial mechanism through lysosomal degradation, the initiation of autophagy has a great impact on the host innate and adaptive immunity. In recent years, a number of studies demonstrated that autophagy can modulate the differentiation of immune cells including neutrophils, T & B lymphocytes via a metabolism pathway (Riffelmacher et al., 2017) (Bhattacharya et al., 2015). Engulfment and Cell Motility protein 1 (ELMO1) of macrophages can also regulate intestinal inflammation induced by infectious agents through an autophagy pathway (Sarkar et al., 2017). Furthermore, autophagy can regulate the secretion of cytokines (including IL-1β, IL-1α, and IL-18) that are activated by the inflammasome. In turn, the sensing of pathogens by pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs) can promote autophagosome formation. Additionally, clearance of pathogens in the cytosol by autophagy may alleviate inflammasome activation. As post-translational modification event, ubiquitination was identified as a core process in pathogenesis and various host inflammatory responses. Ubiquitination is involved not only during autophagy, but also through the inflammatory signaling pathways and the mechanisms of antigen presentation in adaptive immune responses. Salmonella has also evolved some strategies to resist these host immune responses by regulating ubiquitination pathways.

There are numerous defense mechanisms in innate immune system to rapidly recognize and respond to pathogens, including the induction of inflammation which is a critical response to infections. The innate immune system detects pathogens and initiates inflammation through recognition of pathogen-associated molecular patterns (PAMPs) by PRRs, such as TLRs, C-type lectin receptors (CLRs), RIG-I like receptors (RLRs), Nod-like receptors (NLRs), and AIM2-like receptors (ALRs). While TLRs and CLRs are membrane-bound PRRs which detect bacteria in the extracellular space or within endosomes, the rest three receptors are PRRs resided in the cytosol detecting intracellular bacteria (Lamkanfi and Dixit, 2014). Sequestosome 1/p62-like receptors (SLRs) represent new family of innate immune receptors-a category of PRRs engaged in the recognition and capture of intracellular microbes (Figure 3) (Miao et al., 2010; Deretic, 2012). SLRs can initiate autophagy to eliminate intracellular microbes by direct capture and delivery of antimicrobial peptides, and serve as an inflammatory signaling platform. Multiple SLRs, such as p62 and NBR1, recognize either classical or branched Ub chains in association with or in vicinity of cytosolic Salmonellae (Thurston et al., 2009; Wild et al., 2011). A number of Ub recognized proteins, such as galectin 8, TBK1, and LC3, either independently or in combination, may lead to the recruitment of SLRs through their ubiquitin binding domains (UBDs) (Zhou and Zhu, 2015). NDP52 can recognize SLRs and also recruit the Ub tags, having dual functions on elimination of bacteria. It can not only recognize Ub

![Figure 3](image-url)
coated *Salmonella* but also recruit SLRs by the UBDs. Together with additional features of SLRs, it indicated that p62 can promote NF-κB induction and caspase-8 aggregation (Deretic, 2012).

NF-κB and interferon-regulatory factors (IRFs) pathways are two main signaling pathways activated for PRRs to promote pro-inflammatory and antimicrobial transcriptional responses, which play vital roles in inflammatory responses (Kopitar-Jeral, 2015). As a crucial pathway in response to *Salmonella* infection, there are several effectors regulating the inflammatory responses by modulating the NF-κB signaling pathway. In addition to the effectors we have mentioned above, the *Salmonella* virulence factor SrfA can also modulate inflammatory responses by increasing the activation of NF-κB signaling pathway (Lei et al., 2016). In addition, *S. Typhimurium* T3SS effector proteins PipA, GogA, and GtgA can specifically and redundantly target the components of the NF-κB signaling pathway leading to inflammation (Sun et al., 2016). The *Salmonella* T3SS-2 effector protein SpvD inhibits the NF-κB signaling pathway by inhibiting the nuclear transport of NF-κB p65 resulting in systemic growth of bacteria in mice (Rolhion et al., 2016). Remarkably, both NF-κB and IRF pathways are greatly controlled by ubiquitination, and their activation can be affected by several proteins interfering with ubiquitination events (Zhou and Zhu, 2015). Besides its known role as an autophagy receptor, the protein OPTN was previously suggested as a control for the NF-κB and IRF-dependent inflammatory signaling. On one hand, being a homologous to NEMO (NF-κB essential modifier), OPTN competes with NEMO for binding IKKα and IKKβ to be ubiquitinated to inhibit TNF (tumor necrosis factor)-induced NF-κB signaling. On the other hand, the expression of OPTN is affected by NF-κB in response to TNF (Sudhakar et al., 2009). Interestingly, OPTN deficiency in mice reduced the secretion of pro-inflammatory cytokines such as TNF and IL-6, and diminished recruitment of neutrophils into the infected tissue (Chew et al., 2015).

Autophagy is one of the host innate immune responses against *Salmonella*. However, some research indicated that the induction of autophagy has a negative feedback process in inflammatory responses. Deretic et al. observed that the production of IL-1β and IL-18 is increased in the absence of functional ATG16L1 in a mouse model of Crohn’s disease (Marchiando et al., 2013). It has been reported that the inhibition of autophagy was involved in pro-inflammatory cytokines production such as IL-1β and TNF-α in fish (Qin et al., 2016). Furthermore, macrophages infected with *Mycobacterium leprae* present high levels of autophagy activation but lower levels of pro-inflammatory cytokines (Ma et al., 2017). These studies suggested that autophagy induction could affect inflammatory responses, which will fuel advances in studies of *Salmonella* pathogenesis. The presence of specific inflammasome sensors in macrophages, such as NLRP3 and AIM2, could lead to the induction of autophagy. However, autophagosomes can selectively degrade specific inflammasome components, including AIM2, NLRP3, and ASC, etc. (Behnsen et al., 2015). The inflammasomes are intracellular complexes consisting of caspase-1, NLRs such as NLRP3 or NLRC4, and the adaptor molecule ASC (apoptosis-associated speck-like protein containing a CARD), which play a central role in innate immune defense against *S. Typhimurium* (Aachoui et al., 2013b; Man et al., 2014). Activation of inflammasomes are dependent on PAMPs recognition, ATP and intracellular LPS can activate the NLRP3 inflammasome, NLRC4 is activated by flagellin, dsDNA activates AIM2, and toxin-induced modifications of Rho GTPase is the activator of Pyrin inflammasome. Previous studies showed that NLRP3 and NLRC4 recruit ASC and caspase-1 in response to bacterial trigger, and both of NLRC4 and NLRP3 are activated during *Salmonella* infections (Man et al., 2014). Activation of these inflammasomes leads to caspase-1 activation and the secretion of the pro-inflammatory cytokines IL-1β and IL-18, resulting in a form of cell death known as pyroptosis (Figure 3) (Behnsen et al., 2015). Induction of flagellin expression leads to NLRC4-dependent pyroptosis during *S. Typhimurium* infection, which results in the exposure of released bacteria to infiltrating neutrophils (Lage et al., 2013). Man et al. showed that *Salmonella* infections activate a caspase-8-dependent pathway via NLRC4 that induces the formation of an ASC caspase-8-caspase-1 inflammasome complex (Man et al., 2014). A recent study proposed that NLRP3 is also responsible for sensing *Salmonella* through a flagellin-independent mechanism, in which both NLRP3 and NLRC4 trigger ASC-dependent caspase-1 activation. However, the mechanism remains largely elusive (De Jong et al., 2014). Remarkably, the activation of inflammasome NLRP3 is restricted by NF-κB. While macrophages encounter NLRP3-inflammasome activators, mitochondria are damaged and following the release of inflammasome activating signals. On one hand, damaged mitochondria are rapidly ubiquitinated and subsequently eliminated by p62-dependent mitophagy. On the other hand, the mitophagy process restricts the activation of NLRP3. NF-κB playing a core role in this process, stimulating p62 gene transcription resulting in p62-dependent mitophagy and then restrains its own inflammation-promoting activity indirectly (Zhong et al., 2016). In contrast to NLRC4 and NLRP3, NLRP6 and NLRP12 negatively regulate the inflammatory responses during *Salmonella* infections. In addition, Anand et al. found that mice deficient in NLRP6 exhibit lower *Salmonella* burden in organs, as well as an increase in the phosphorylation of IκB, indicating that this is related to the activation of NF-κB signaling (Anand et al., 2012; Zaki et al., 2014). However, the mechanisms by which *Salmonella* activates NLRP6 and NLRP12 remain to be determined.

The ubiquitination pathway and autophagy also have profound effects on innate immune responses through other mechanisms. The poly-Ub K63 chains have been involved in cell responses to danger signals through the TLRs, the interleukin-1 receptor (IL-1R) and the TNF (Ligeon et al., 2011). It was demonstrated that K63-linked Ub chains could mediate protein substrates targeting to the autophagy or ubiquitination pathway. The TNF receptor-associated factors (TRAF) family Ub ligases catalyze the formation of K63-linked Ub chains that activate NF-κB, ERK, JNK, and other signaling pathways (Yazlovitskaya et al., 2015). NOD2 (nucleotide oligomerization domain 2) and cytosolic pattern recognition receptors regulate the formation of K63-linked poly-Ub chains on the NEMO, thereby leading to activation of IKK and NF-κB pathway (Watanabe et al.,
TRIM38, an E3 Ub ligase, can regulate TLR3/4-mediated innate immunity and inflammatory responses. This protein catalyzed K48-linked poly-ubiquitination of the TLR3/4 adapter protein TIR domain which contains adapter-inducing IFN-β and promoted its proteasomal degradation in immune cells (Hu et al., 2015). As a pivotal NF-κB suppressor under TLRs, A20 can be captured by autophagy receptor p62 and then be eliminated in the autophagosome, thus enhancing NF-κB activity (Kanayama et al., 2015). Moreover, Park et al. found that p62 is required for TNF and IL-1β production mediated by NOD2 (a cytosolic PRR) and up-regulates its signaling response through NF-κB activation. In contrast, p62 can induce MyD88 aggregation to suppress TLR signaling cascades (Park et al., 2013). LLRC25, a member of the LRR-containing protein family, revealed a new crosstalk between inflammation and autophagy. LLRC25 can promote the degradation of p65/RelA through autophagy, acting as an inhibitor of NF-κB signaling pathway (Feng et al., 2017).

Upon infection, Salmonella can not only trigger the autophagy flux in host cells but also evolve mechanisms for inhibiting this response. When T3SS-1 inflicted SCVs damage at the early stages of S. Typhimurium infection leading to bacterial exposure to the cytosol and initiating autophagy, autophagy promote repair of T3SS-1 inflicted damage to SCVs membrane conversely. Collectively, autophagy recognition of the damaged SCVs leads to bacterial elimination as well as SCVs repair. The balance between them relies on specific host responses and coordinate expression of bacterial virulent factors (Kreibich et al., 2015). Salmonellae recruit FAK (the host tyrosine kinase focal adhesion kinase) to the SCVs in a SPI-2 dependent way and then FAK suppresses autophagy, thereby preventing cell autonomous elimination and restrain the activation of innate TRIF-dependent type I interferon immune responses (Owen et al., 2016). The linear Ub chain assembly complex (LUBAC) was recently identified as a critical modulator of both innate immunity and inflammation signaling. LUBAC consists of SHANK-associated RH-domain–interacting protein (SHARPIN), heme-oxidized IRP2 Ub ligase-1 (HOIL-1), and HOIL-1–interacting protein (HOIP). Components of LUBAC were identified to control TLR3-mediated innate immunity (Zinggrebe et al., 2016). The M1 (methionine)-linked linear poly-Ub chain, synthesized by the E3 ligase LUBAC, serves as a novel signaling platform. Once coated with this platform, S. Typhimurium could be transmuted into antibacterial and pro-inflammatory status and recruit OPTN as well as NEMO to initiate autophagy process and NF-κB signaling response through STAT3 (Signal Transducer and Activator of Transcription 3) phosphorylation, a conductive signaling event in response to Salmonella. Upon exposed in cytosol, Salmonellae were Ub coated and following the recruitment of HOIP, HOIL, and Sharpin. The combination of HOIP, HOIL, and Sharpin is termed as LUBAC, which can synthesize the M1-linked Ub chains. Ultimately, the M1-linked linear poly-Ub coated on Salmonellae recruit OPTN and NEMO to initiate autophagy and NF-κB signaling pathways, which play crucial roles in restrict proliferation of Salmonellae independently. OTULIN is a DUB which inhibits the recruitment of NEMO and ultimately NF-κB pathway by restraining the formation of M1-linked Ub chains.

The inflammatory responses to Salmonella infection are a complex process, involving the common recognition of PAMPs by PRRs as well as autophagy and ubiquitination mechanisms. There are numerous strategies for the host to clear infections remain to be a threat to the public health owing to its transmissibility and pathogenicity. Despite copious amounts of advances in the understanding of autophagy and ubiquitination involved in responses to pathogens, how these pathways and immune responses need to be further studied. A novel strategy of ubiquitination-dependent mechanism for Salmonella to manipulate host cells has also been revealed. S. Typhimurium T3SS effectors SopB and SopE2 can trigger ubiquitination of TRAF6, which is involved in STAT3 (Signal Transducer and Activator of Transcription 3) phosphorylation, a conductive signaling event in response to S. Typhimurium infection (Figure 3) (Ruan et al., 2017).

The inflammatory responses to Salmonella infection are a complex process, involving the common recognition of PAMPs by PRRs as well as autophagy and ubiquitination mechanisms. There are numerous strategies for the host to clear Salmonellae, while the bacteria also evolves mechanisms to subvert the host defenses. As reviewed in details of autophagy, ubiquitination and related inflammatory responses, a comprehensive understanding of host-Salmonella interaction was presented.

SUMMARY AND FUTURE DIRECTIONS

Salmonella infections remain to be a threat to the public health owing to its transmissibility and pathogenicity. Despite copious amounts of advances in the understanding of autophagy and ubiquitination involved in responses to pathogens, how these
processes are regulated to counteract Salmonella is incompletely defined. We therefore focus on autophagy and ubiquitination to clarify and summarize the host-pathogen interactions in detail. The related inflammatory responses have also been illuminated due to its closely association with the process of autophagy and ubiquitination pathways during bacterial infection. Since many bacteria have evolved strategies that allow for evasion of the autophagy pathway and host immune responses, it is important to identify the mechanism by which Salmonella inhibits autophagy and subverts the host responses. In the last ten years, more and more studies illuminate the interaction between Salmonella T3SS effector proteins and the host Ub pathways. Increasing evidence suggests that diverse mechanisms including molecular mimicry, formation of novel structures, and new enzymatic activities of effectors are involved in a crosstalk, leading with a modulation of autophagy and ubiquitination pathways. In this review, we summarized the connection between the Ub-proteasome system with autophagy and inflammatory responses in the context of Salmonella infection, in particular, the way effector proteins regulate the host defense by Ub and autophagy. The future challenge will be to address the commons of all the effectors, which may be helpful for eliminating Salmonellae in more efficient ways.

AUTHOR CONTRIBUTIONS
RH and SW: outlined the manuscript; LW, JY, and HN: wrote the manuscript; LW, RH, and SW: edited the manuscript.

FUNDING
This work is supported by grants from the Natural Science Foundation of China (No. 81471572, No. 81671976, No. 31670140). It was also supported by a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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